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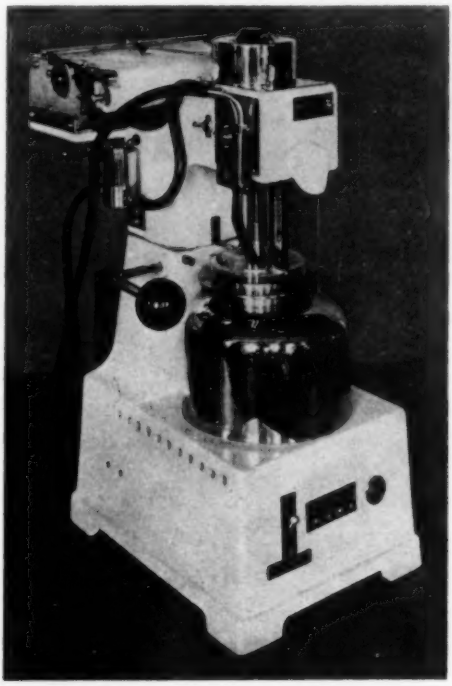

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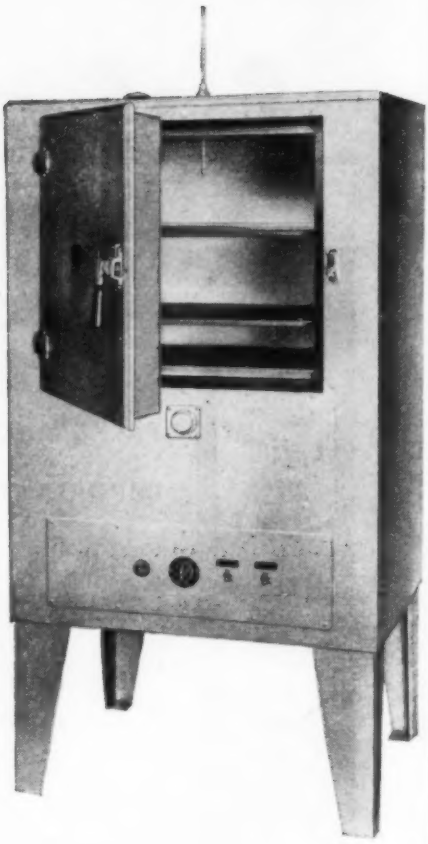

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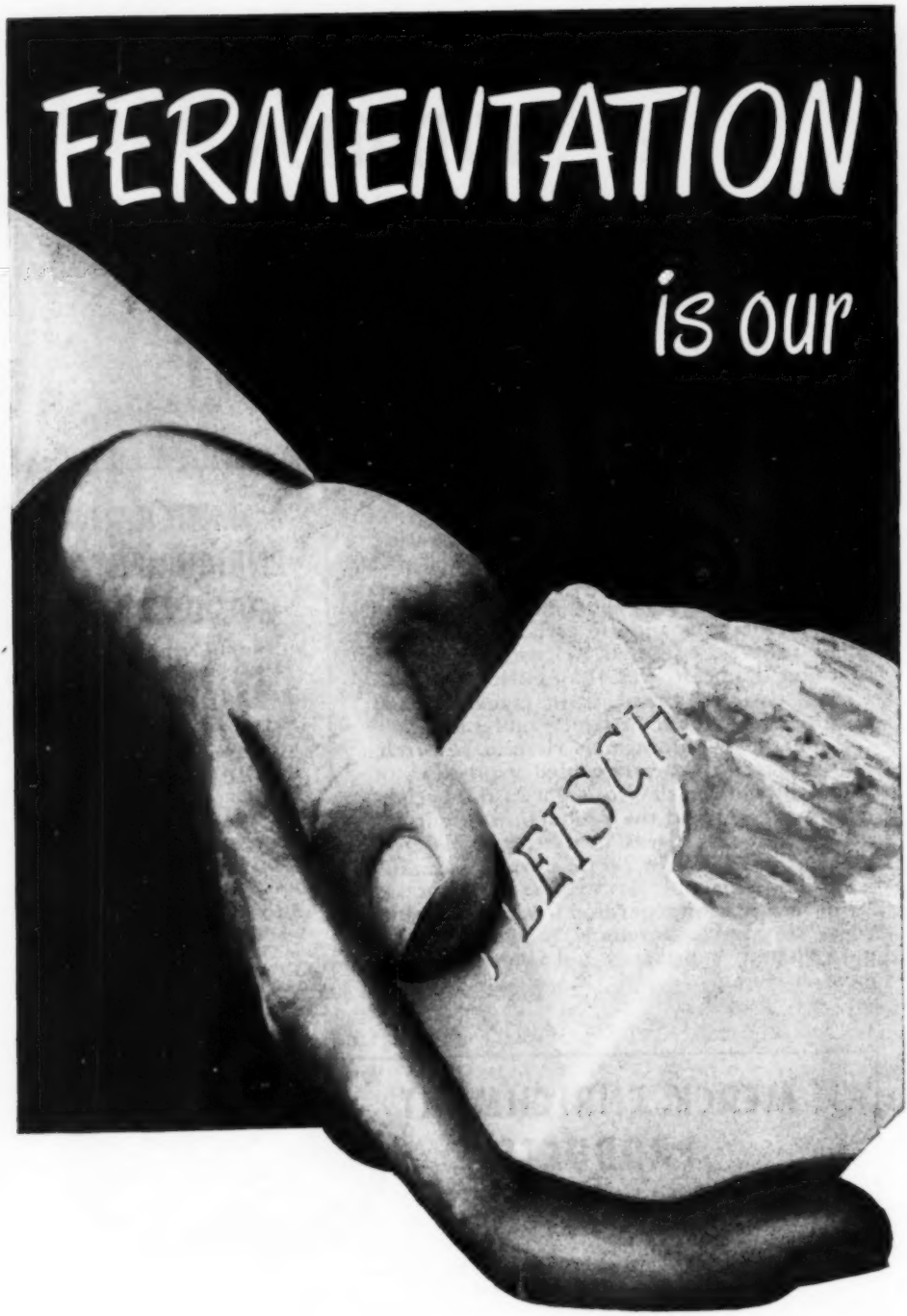
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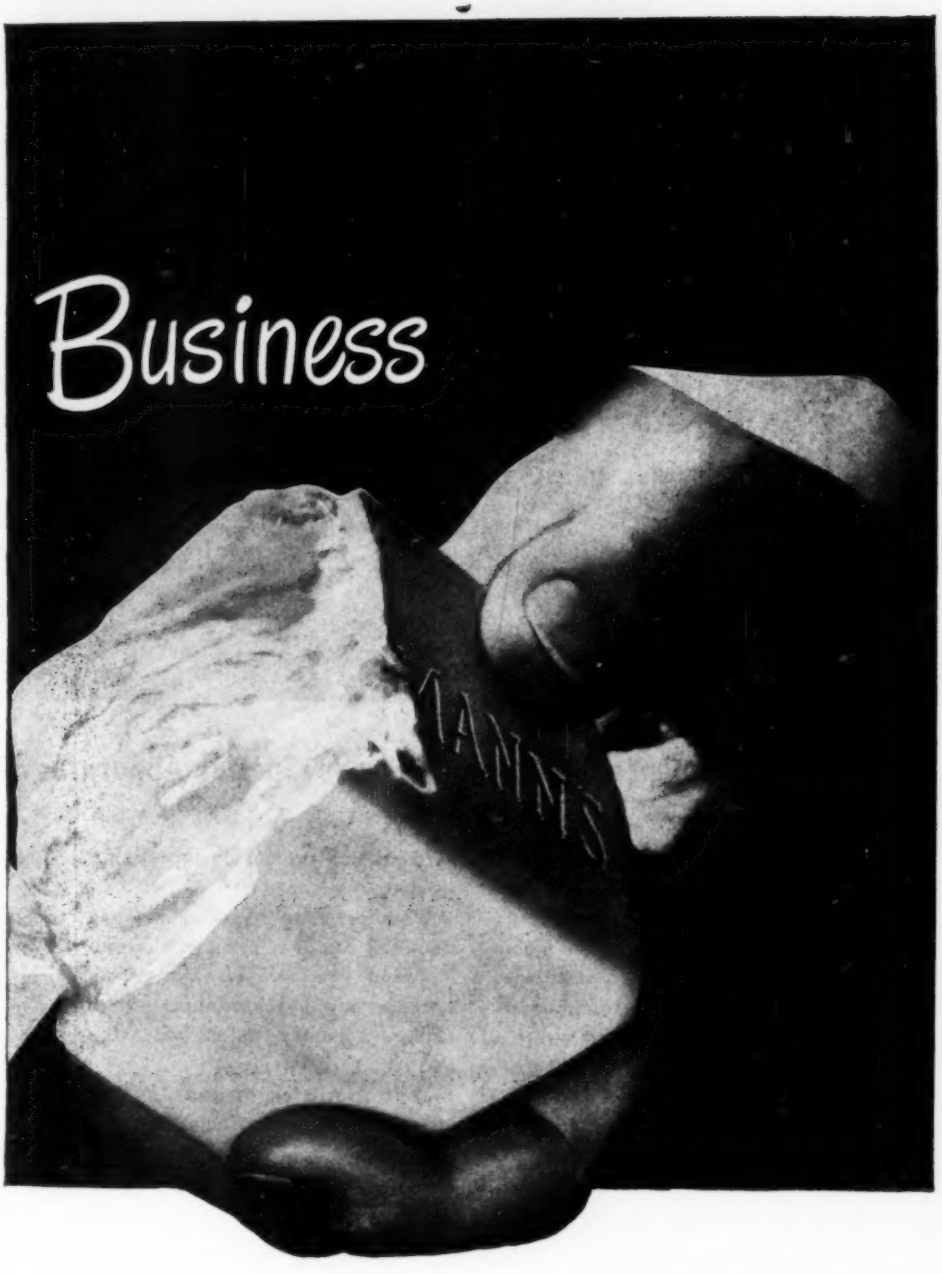

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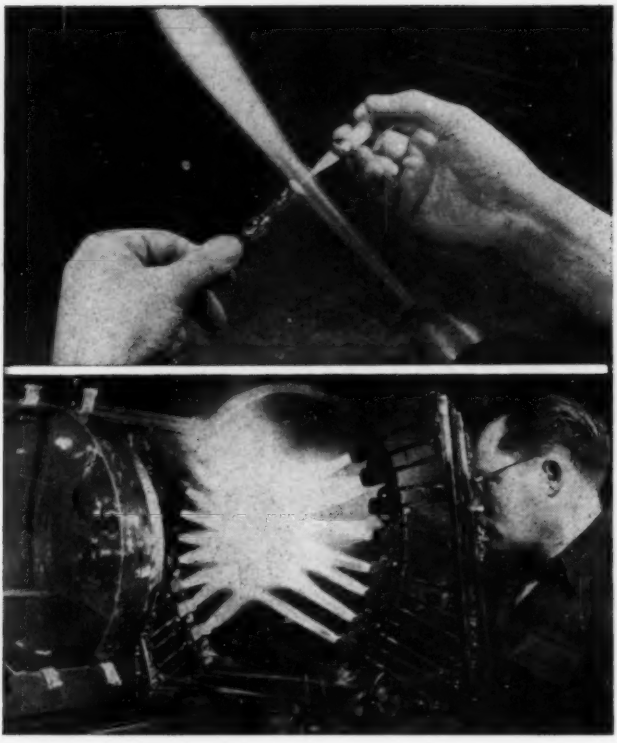

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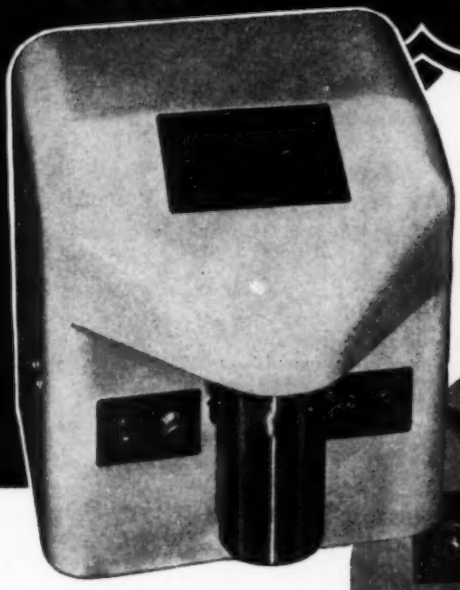

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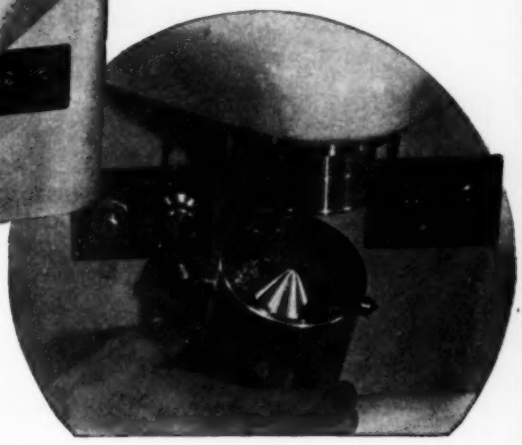

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CEREAL CHEMISTRY

VOL. XXVI MAY, 1949 No. 3

EFFECT OF HEAT TREATMENT OF SEPARATED MILK ON THE PHYSICAL AND BAKING PROPERTIES OF DOUGHS ENRICHED WITH DRY MILK SOLIDS [1]

ROBERT A. LARSEN,[2] ROBERT JENNESS,[3] and W. F. GEDDES [4]

ABSTRACT

The three major components of nonfat dry milk solids, casein, lactose, and the milk serum proteins, were heated in solution for 30 minutes at 70°C., 75°C., and 80°C., and incorporated into doughs in amounts equivalent to the addition of 6% of nonfat milk solids. The unheated fractions all depressed loaf volume, with lactose having the greatest effect. In the absence of potassium bromate, heat treatment improved the baking quality of the milk serum proteins considerably but had no effect on the baking behavior of casein or lactose. Inclusion of bromate in the baking formula increased the loaf volume when unheated milk serum proteins were added to doughs; bromate also somewhat improved the baking results with lactose.

Farinograms and extensograms for salt-water doughs showed that the inclusion of either nonfat milk solids prepared from raw separated milk or unheated serum proteins caused marked softening upon prolonged mixing, greatly increased extensibility, and a lowered resistance to extension. Heat treatment overcame these effects.

Improvement in the baking quality of separated milk is a function of both the time and temperature of heating. The minimal conditions for maximum improvement were found to be 73°C. for 30 minutes, about 85°C. for 7 minutes, and 92°C. for the flash method (about one minute) of heating.

Several studies have been made in these laboratories of the factors that influence the baking quality of nonfat milk solids since the pioneer work of Greenbank *et al.* (4) and of Grewe and Holm (5) showed that heat treatment of separated milk prior to drying improves the baking quality of the resulting nonfat milk solids. Skovholt and Bailey (11) demonstrated that heat treatment of separated milk reduces the tendency of doughs to slacken upon extended mixing. The farinograph

[1] Manuscript received December 28, 1948. Presented at the Annual Meeting, May, 1948. Paper No. 2312, Scientific Journal Series, Minnesota Agricultural Experiment Station. Some of the data in this paper were taken from a thesis presented to the Graduate School of the University of Minnesota by R. A. Larsen in partial fulfillment of the requirements of Doctor of Philosophy, June, 1947.

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studies of Stamberg and Bailey (13) indicated that the dough-softening factor is concentrated in the whey portion of milk, and is heat-labile. Employing the Heyrovsky micropolarograph, they found that fresh separated milk exhibited a wave characteristic of sulfhydryl compounds, whereas boiled milk failed to show this wave. Sulfhydryl compounds are known to cause dough softening and as the sulfur content of the serum proteins is much higher than that of casein (Baernstein, 1), these observations, although unconfirmed by baking tests on the milks or milk fractions, suggest that the heat improvement of separated milk for breadmaking primarily involves the serum proteins and probably is associated with a decrease in the amount or activity of sulfhydryl groups in these proteins.

It appeared logical to subject separated milk, and fractions prepared from it, to a series of controlled heat treatments and to study the baking effects and the biochemical properties of the various fractions. Should it be established that the improvement is associated with certain biochemical changes in a particular fraction, a simple test for production control purposes might evolve. Such a development would be of considerable practical significance since baking tests are empirical and time consuming, and the vast majority of producers of nonfat milk solids do not have the facilities for carrying them out. During the progress of this research, Harland *et al.* (6) have reported baking experiments undertaken with a similar objective.

The present paper deals with the influence of heat treatment of casein, serum protein, and lactose on their effects on loaf volume and on the physical properties of doughs. The relation of time and temperature of heating skim milk to the baking behavior of nonfat milk solids prepared from it was also investigated.

Materials and Methods

Fresh separated milk produced from bulked milk of the University dairy herd was used throughout this study with the exception that the series of flash-heated samples was supplied by a commercial organization.

Milk Fractions. Since heat treatment of milk results in the partial precipitation of the milk serum proteins with the casein upon subsequent fractionation (7, 10), it was necessary to make the protein separations prior to heat treatment to secure pure fractions.

Casein was prepared from the separated milk by isoelectric precipitation according to the method of Van Slyke and Baker (17) and purified by washing, redispersion in 5% ammonium hydroxide solution to pH 6.6, reprecipitation, and washing. For the purpose of heat treatment, the purified casein was reconstituted as a calcium phos-

phocaseinate solution. This was accomplished by triturating 2.5 g. casein, 51 mg. hydrated trisodium citrate, 100 mg. hydrated tripotassium citrate, and 50 mg. calcium hydroxide in a mortar. Seventy-five milliliters of clear saturated calcium hydroxide solution were slowly added followed by the dropwise addition, with thorough mixing, of 13.25 ml. of 1.0% orthophosphoric acid solution containing 0.3% calcium hydroxide and 0.635% monocalcium citrate. The solution was then made up to 100 ml., yielding one of the same caseinate concentration and pH as milk.

The milk serum protein fraction for the baking tests was prepared from fresh raw separated milk at 25°C. (or 35°–40°C. in some cases) by adding 100 ml. of 10% acetic acid solution per liter, holding for 15 minutes, and adding 100 ml. of 1.0 *N* sodium acetate solution per liter. After 10 minutes the precipitated casein was removed by filtering or centrifuging. The serum was dialyzed free of water-soluble constituents and buffered with phosphate buffer to a pH of 6.6 and ionic strength of 0.1 in the final solution (adjusted to a protein concentration of about 0.7 g. per 100 ml.).

The milk serum protein for the physical dough tests was prepared by precipitation of the casein from fresh skim milk by adjusting the pH to 4.6 with 4% hydrochloric acid solution, followed by dialysis[5] of the serum against distilled water. Aliquots of this solution were adjusted to a concentration of 0.7 g. of protein per 100 ml. with distilled water and heat-treated as described in the next section.

Heat Treatment. Heat treatments of skim milk and solutions of calcium phosphocaseinate, serum protein, and lactose[6] were carried out at 70°, 75°, and 80°C. in a thermostatically controlled water bath. The preparations were held in the bath for 30 minutes after they had attained the selected temperature and then rapidly cooled by running water.

Concentration of the preparations was effected by pervaporation in Visking sausage casings. Some of them were dried from the frozen state under vacuum in a chamber containing alumina for a desiccant.

Nonfat Milk Solids. Three lots of spray-dried nonfat dry milk solids were prepared from bulked separated milk which had been preheated by different methods. For the first series, sublots from one source were heated in a steam-jacketed vat for 30 minutes at temperatures ranging from 63°C. to 85°C. The lots of the second series were heated in a similar manner for 7 minutes at temperatures varying from 68°C. to 82°C. The third series was flash-heated in Grey-Jensen

[5] This dialysis was not exhaustive. The preparations contained about 75% protein on the dry basis. The milk serum protein for the physical dough tests was prepared some months prior to that used for baking. The precipitation of the casein with acetate buffer is more convenient than with hydrochloric acid, but the two methods yield serum protein of identical characteristics.

[6] A solution containing 6 g. of U.S.P. lactose per 40 ml. was used throughout these studies.

equipment at a series of temperatures from 77°C. to 110°C. All samples were spray dried in conventional type driers, packed under nitrogen in cans, and stored at −5°C. until used in baking studies.

Baking Tests. Several studies have shown that the inclusion of nonfat milk solids in the baking formula increases the dough-mixing requirements, the fermentation tolerance, and also the bromate requirement and bromate tolerance; moreover, the effect of nonfat milk solids on bread quality is influenced by the characteristics of the flour.

A short study was undertaken with two commercial samples of nonfat milk solids, one of high and the other of low quality, to develop a suitable formula and baking procedure for evaluating the samples of nonfat milk solids and milk fractions. The variables which were investigated included flour strength, baking method (straight dough vs. sponge and dough), percentage of nonfat milk solids, bromate level, and severity of punching. The best differentiation between the nonfat milk solids of good and poor quality was obtained with a very strong bakers' patent hard red spring wheat flour (14.5% protein on a 14.0% moisture basis), the addition of 12% of nonfat milk solids and 2.0 mg. of potassium bromate per 100 g. of flour to the ingredients of the A.A.C.C. basic formula, a mixing time of 3 minutes (Hobart Swanson mixer), a 2-hour fermentation, and a single passage of the dough through a National dough sheeter (set at 9/16 inch) at the time of molding. The baking tests in the present study, however, were conducted mostly with 6.0% of nonfat milk solids or their equivalent to reduce the labor involved in preparing the fractions. In most cases, also, a mixing time of 2.5 minutes was used. Each sample was baked at least in duplicate with 0, 1, and 2 mg. of potassium bromate respectively per 100 g. of flour and the standard errors of the loaf volumes were calculated for each series to aid in interpreting the data. By this baking procedure, commercial nonfat milk solids of poor quality gave loaf volumes averaging about 10% lower than those obtained when nonfat milk solids of good baking properties were used.

Physical Dough Tests. Farinograms and extensograms were made at 30°C. with salted doughs containing 12% of nonfat milk solids or equivalent amounts of the various fractions (4.3% of casein, 0.9% of serum proteins, or 6.0% of lactose). Two per cent of sodium chloride was used and the proper amount of water was added to the heat-treated products to give a maximum dough consistency of 500 units. The absorption determined for each heat-processed material was employed in the farinogram test on the corresponding untreated samples. The total weight of dough was adjusted in all cases to 480 g. as recommended by Stamberg and Merritt (14). For the extensograph tests, doughs were mixed in the farinograph as already described, rounded

and molded in the extensograph, and then placed in the dough trays where they were held at 30°C. for 45 minutes and an extensogram made. Two additional extensograms were made on the same dough at successive intervals of 45 minutes as described by Merritt and Bailey (8).

Results

Effect of Milk Fractions and Their Heat Treatment on Loaf Volume. The effects of the three milk fractions, with and without heat treatment, on loaf volume are shown in Table I. Casein, milk serum proteins, and lactose all depress loaf volume, with the last-mentioned having the greatest effect. In the absence of bromate, heat treatment improved the baking quality of the milk serum proteins considerably but had no effect on the baking quality of the other two fractions.

TABLE I

EFFECT OF HEAT TREATMENT OF CASEIN, MILK SERUM PROTEIN, AND LACTOSE ON LOAF VOLUME OF BREAD BAKED AT VARIOUS BROMATE LEVELS

(Amount of each fraction per loaf equals that present in 6 g. of nonfat milk solids)

Milk constituent	Potassium bromate[1]	No. of replicates	Mean loaf volume[2] for samples heated for 30 minutes			
			Unheated control	70°C.	75°C.	80°C.
	mg.		*cc.*	*cc.*	*cc.*	*cc.*
Nil (control)	0	12	736	—	—	—
	1	6	738	—	—	—
	2	4	712	—	—	—
Serum protein	0	3	602	663	695	700
	1	2	712	760	742	695
	2	2	728	745	750	720
Casein	0	5	696	661	692	674
	1	4	702	682	718	674
	2	2	735	695	738	715
Lactose	0	3	597	626	645	642
	1	2	670	678	692	685
	2	2	690	702	685	695

[1] Quantity of potassium bromate per 100 g. of flour.
[2] Standard error of a single determination equals 27.6 cc.

When bromate was used, the loaf volume of the bread baked with the milk serum protein fraction increased considerably especially when this fraction had not been heated before drying. Bromate also somewhat improved the baking results with lactose. Several investigators have noted that the inclusion of nonfat milk solids in a baking formula increases the bromate requirement of a dough (2, 9, 11). It now ap-

pears that the milk serum proteins and, to a small extent, lactose account for this effect.

The lower loaf volume of bread when the three milk fractions were added singly led to a series of baking tests designed to measure the effect on loaf volume of various combinations of the fractions (Table II). These were added to the dough formula in the amounts which would have been present had 6% of nonfat milk solids been used. A comparison of the loaf volumes in Tables I and II shows that the depressions caused by the various combinations are less than those which

TABLE II

EFFECT OF HEAT TREATMENT OF MILK CONSTITUENTS ON THE LOAF VOLUME OF BREAD WHEN BAKED IN COMBINATION AT VARIOUS BROMATE LEVELS

(Fractions heat treated before combining and added in amounts per loaf equal to those present in 6.0 g. of nonfat milk solids)

Milk constituent	Potassium bromate[1]	No. of replicates	Mean loaf volume[2] for samples heated for 30 minutes			
			Unheated control	70°C.	75°C.	80°C.
	mg.		*cc.*	*cc.*	*cc.*	*cc.*
Casein+serum protein	0	4	601	646	642	618
	1	3	615	672	650	583
	2	2	635	708	680	635
Lactose+serum protein	0	3	582	640	642	672
	1	2	670	732	742	698
	2	2	688	680	702	665
Casein+lactose	0	3	662	662	683	642
	1	2	712	685	665	655
	2	2	708	695	688	680
Casein+lactose+serum protein	0	5	595	647	641	623
	1	2	645	658	672	645
	2	2	645	680	685	665

[1] Quantity of potassium bromate per 100 g. of flour.
[2] Standard error of a single determination equals 24.5 cc.

would be expected from the additive effects of the individual fractions. Whenever the milk serum proteins are present, the improving effect of heat treatment is apparent, again demonstrating the importance of this fraction in the heat-induced reaction.

Since the fractions were heat treated after their isolation, the question arises whether the conclusions concerning the differential effects of the treatment on loaf volume are applicable to separated milk itself. Rowland has shown that heat treatment of milk at 80°C. for 30 minutes results in the precipitation of most of the serum proteins

along with the casein upon subsequent acidification (10). An interaction between the serum proteins and casein may possibly occur upon the heat treatment of milk which might result in a different effect on baking properties than that obtained in these and other studies (6) where the heat treatments were applied to various fractions after their isolation.

To investigate this possibility, casein and the milk serum protein fraction which had been prepared in the manner already described were combined to yield a solution containing the same concentrations of these proteins as separated milk; aliquots of this solution were heat

TABLE III

EFFECT OF MILK CONSTITUENTS HEATED TOGETHER COMPARED TO HEAT-TREATED NONFAT MILK SOLIDS ON THE LOAF VOLUME OF BREAD

(Amount of each fraction per loaf equals that present in 6 g. of nonfat milk solids)

Material heat treated	Potassium bromate[1]	No. of replicates	Mean loaf volume[2] for samples heated for 30 minutes			
			Unheated control	70°C.	75°C.	80°C.
	mg.		*cc.*	*cc.*	*cc.*	*cc.*
Control (No milk constituents)	0	12	736	—	—	—
	1	6	738	—	—	—
Casein+serum protein	0	2	602	702	705	710
	1	2	618	705	708	698
Casein+lactose+serum protein	0	2	588	648	658	678
	1	2	610	660	688	672
Skim milk	0	2	570	582	678	672
	1	2	588	590	638	648

[1] Quantity of potassium bromate per 100 g. of flour.
[2] Standard error of a single determination equals 24.3 cc.

treated at different temperatures, and the solids isolated. Similarly, casein, serum proteins, and lactose were heated in combination so that the quality of this synthetic milk after drying could be compared with that of nonfat milk solids which had been prepared directly from skim milk subjected to similar heat treatments. The loaf volumes are summarized in Table III.

Heat treatment markedly improved the baking quality of the combinations, and any interaction of casein and serum protein during heating does not interfere with the baking behavior of the latter fraction. In fact, when lactose was also included, the baking results for the mixture were so similar to those for nonfat milk solids that it may

be concluded that these three fractions fully account for the influence of nonfat milk solids in breadmaking.

With the flour and baking procedure employed in these tests, the inclusion of nonfat milk solids in the baking formula lowers the loaf volume. This is contrary to the majority of reports in the literature. However, most investigators have used shortening in their formulas, and E. G. Bayfield [7] has found that improvement in loaf volume is only obtained upon the addition of milk solids if the formula also contains shortening. This observation has been confirmed by the authors, but the use of shortening did not increase the relative differences in loaf volume obtained with fractions which were subjected to different heat treatments. In view of this and the desirability of limiting the number of variables in the baking test to the essential minima, shortening has not been used in these studies.

The finding that the baking improvement obtained by heat treating separated milk is associated with the serum protein fraction is in agreement with the findings of Harland, Ashworth, and Golding (6) which were published while the studies reported here were in progress. They found that the baking properties of dialyzed acid and rennet "wheys" prepared from skim milk were improved by heat treatment, whereas casein prepared by the acid method was not benefited; also, heat treatment had practically no effect on the dialyzable portion of the milk.

Physical Dough Tests. Typical farinograms and extensograms illustrating the effect of heat treatment of skim milk and its fractions on the physical properties of doughs are reproduced in Fig. 1. Curves are included for doughs made without milk and also for commercially prepared nonfat milk solids known to be of good and poor quality.

The farinograms show that the unheated milk serum proteins caused marked softening of dough upon extended mixing, a property which was greatly reduced by heat treatment; casein also had a slight softening action. The similarity between the curves for unheated serum proteins and laboratory-dried nonfat milk solids prepared from unheated separated milk and those for these preparations when heat treated is very striking. There was little difference between the plastic properties of the control dough and of those made with nonfat milk solids of high quality.

The extensograms for doughs made with nonfat milk solids prepared from unheated separated milk and of unheated serum proteins are characterized by very great extensibility and low resistance to extension. Heat treatment, however, yields extensograms which are very similar

[7] Private communication concerning unpublished studies at Kansas State College, Manhattan, Kansas. Present address Standard Milling Company, Chicago, Illinois.

to those for the dough made without milk. The differences between the extensograms for nonfat milk solids of good and poor baking quality are more striking than between the corresponding farinograms and it would appear that the extensograph would serve as a useful index of the quality of nonfat milk solids for breadmaking.

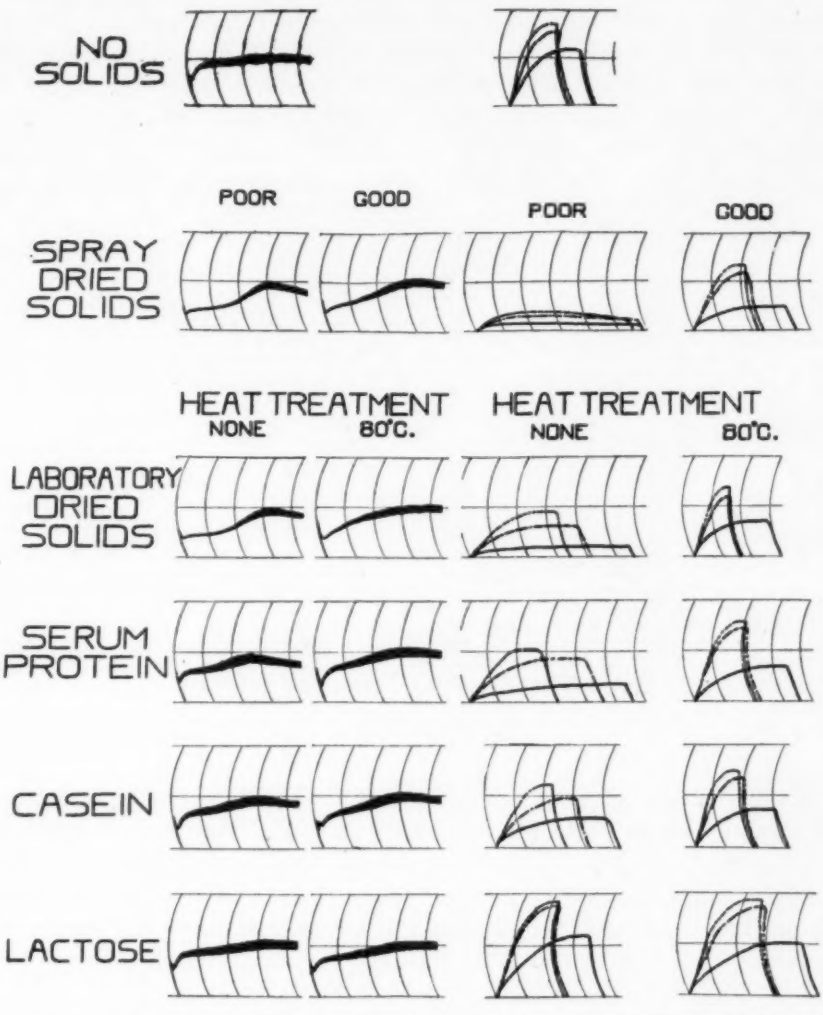

Fig. 1. The effect of nonfat milk solids and fractions thereof on the physical properties of a salt-water dough as measured by the farinograph and extensograph. The extensograms for a rest period of 45 minutes are shown by a solid line; those for a 90-minute rest period by a dot-dash line; and those for a 135-minute rest period by a dot-dot line.

Relation of Time and Temperature of Heating to Baking Quality. The loaf volumes obtained for doughs containing 12% of nonfat milk solids prepared from defatted milk which had been preheated at vari-

ous temperatures and times are shown in Fig. 2. For the flash-heat treatments and that conducted for 30 minutes, the improvement in loaf volume to a maximum occurred within a rather narrow temperature range; further increases in temperature had little if any effect on loaf volume. Unfortunately, the equipment available for the 7-minute heat treatment did not have sufficient capacity to obtain temperatures higher than 85°C., but it appears from the other curves that a slightly higher temperature may be necessary to obtain maximum improvement. The standard error of the mean values recorded in Fig. 2 is 10.5 cc. and the apparent decreases in loaf volume in two of the series when the milks were preheated at high temperatures are not statistically significant. The minimum temperature required for maximum

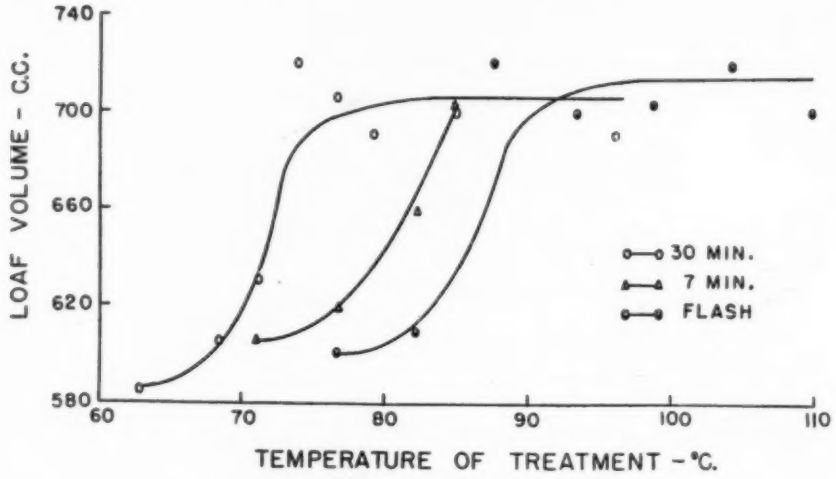

Fig. 2. Effect of time and temperature of preheat treatment on the baking properties of nonfat milk solids.

loaf volume increased with a decrease in heating time, being 73°C. for 30 minutes, about 85°C. for 7 minutes, and 92°C. for the flash method (about one minute). These treatments may be considered as the minimum necessary in the commercial production of nonfat milk solids of good baking quality. Higher temperatures might be desirable provided browning of the solids does not occur.

Discussion

These researches fully confirm the belief of Stamberg and Bailey (13) and of Harland, Ashworth, and Golding (6) that the improvement in the quality of separated milk for breadmaking which is brought about by heat treatment is associated with biochemical changes in the serum proteins. In addition, it has been found that casein and lactose

also lower loaf volume, an influence that is independent of the heat treatment. The action of casein can be explained as being due to the formation of a complex with gluten, thereby lowering its strength, but the role of lactose is not so readily explained. When this sugar was added to doughs in an amount equivalent to that present in 12% of nonfat milk solids no definite evidence was obtained that it had any influence on either gas production or gas retention. The farinograph tests indicate that lactose slightly increases dough consistency and that its harmful effects in baking might have been less pronounced if higher absorptions had been used.

Nonfat milk solids contain 36% casein, 50% lactose, and 7.5% serum proteins. It is significant that the serum proteins, which are present in such a low percentage, have the greatest influence on dough consistency, dough extensibility, and resistance to extension, and are responsible for the improvement in the baking quality of defatted milk upon heat treatment. The fact that the milk serum proteins are much richer in sulfur than casein and cause a decrease in dough consistency similar to the addition of small quantities of sulfhydryl compounds, such as cysteine and glutathione (2, 3, 12, 15), led Stamberg and Bailey (13) to suggest that the dough-softening action of raw milk is caused by the sulfhydryl groups of cysteine and that upon heat treatment these groups are either oxidized to the more stable disulfide linkage or are masked by heat treatment. Their polarographic studies indicated that sulfhydryl groups disappeared when milk was heated. It is noteworthy that nonfat milk solids increase the quantity of potassium bromate required for optimum baking results. This was found to be true also for milk serum proteins.

The fact that improvement in the baking quality of defatted milk is a function of both the time and temperature of heating indicates that heat denaturation of the serum proteins is the fundamental biochemical reaction involved. Protein denaturation may cause a molecular rearrangement in which many of the sulfhydryl groups become less available for reaction, a mechanism which would have the same effect as oxidation. A study of the effect of heat on the biochemical properties of the milk serum proteins is in progress.

Acknowledgments

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AN EVALUATION OF CABINET FERMENTATION WITH COMMERCIAL SIZE SPONGES AND DOUGHS [1]

G. GARNATZ,[2] P. W. HODLER,[3] and W. ROHRBAUGH [4]

ABSTRACT

The fermentation of sponges in open troughs and in a three-trough capacity cabinet under actual commercial shop conditions was studied. This included tracing the expansion of the sponges, observing dough-room temperature, cabinet temperature, and sponge temperature increase, and the concentration of carbon dioxide in the atmosphere within the cabinet and above the cabinet sponges. An adaptation of the pressuremeter test was applied to compare the fermentation characteristics of cabinet and open-trough sponges, and crumb compression measurements were made on some of the resultant bread.

Traffic in and out of the type cabinet employed nullifies any attempt to "condition" the cabinet with respect to the carbon dioxide content of its atmosphere. The maximum carbon dioxide concentration attained in any part of the cabinet is approximately 20%. This was attained when a sponge time of 4 hours 30 minutes was employed. It is possible that, with a shorter sponge time, traffic would tend noticeably to reduce the carbon dioxide content.

When sponges at or near maximum rise completely fill the trough, the concentration of carbon dioxide over the sponge does not exceed 18%. If the size of the sponge is reduced so that at maximum rise, the sponge is still well below the top edge of the trough, the carbon dioxide concentration over the sponge builds up to as much as 37%.

Spring wheat flour apparently gives lower carbon dioxide concentrations than does hard winter wheat flour.

The cabinet and open-trough sponges rise at the same rate and to the same height. The progress of fermentation is apparently no different in the cabinet sponges from what it is in the open-trough sponges. Cabinet fermentation does not reduce sponge time, nor does it permit the use of greater absorption in the dough stage. The doughs from the cabinet sponges, for all practical purposes, machine no better than doughs from sponges fermented in open troughs.

The cabinet is effective in insulating the sponges against significant fluctuations in dough-room temperature. It usually maintains a slightly higher temperature than the dough room, and automatically provides sufficient humidity to prevent crusting of the sponges.

The bread resulting from the sponges fermented in the cabinet is, for practical purposes, no better in quality than the bread made from the sponges fermented in the open troughs, despite the higher carbon dioxide concentrations found over the cabinet sponges. The bread from the cabinet sponges is no softer than, and stales no differently from, the bread made from the open-trough sponges.

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[4] The Zim Bread Company, Colorado Springs, Colorado.

In the February 28, 1942, issue of *Baker's Helper*, there was announced a method of fermenting sponges in a restricted space. This method has since been designated "cabinet fermentation." In the original article, it was represented that resort to cabinet fermentation reduced sponge time to about 2 hours 15 minutes; permitted the sponges to develop more expansion; resulted in 2% extra absorption in the dough; and, in general, yielded bread of better quality.

Since then, there has been much discussion of the process. Many of the observations reported have been lacking in objectivity, but at least three well-organized studies have been published thus far.

Johnson (3) by means of laboratory-scale baking tests found that the rate of expansion and the height of sponge were not affected by the atmospheric conditions involved in cabinet fermentation. He did report that, in an atmosphere containing between 15.5 to 25% carbon dioxide and with a sponge time of 3 hours, the bread produced was better than the control fermented in normal air. No effect on dough absorption was mentioned in this study; but it may be inferred that better handling qualities were noted in the doughs made from cabinet-fermented sponges.

Schoonover, Freilich, and Redfern (4) compared cabinet sponges and noncabinet sponges under varying conditions of dough-room temperature and humidity, sponge temperature, and time, as well as the amount of yeast. They concluded that cabinet fermentation is of no value under controlled conditions of temperature and humidity in which the room temperature is equal to, or greater than, the temperature of the sponge as it is discharged from the mixer. When the dough-room temperature was much lower than the starting temperature of the sponge, cabinet fermentation showed definite advantages over open-trough fermentation. They found that most of the doughs from the cabinet sponges machined better. It was concluded that the effectiveness of cabinet fermentation depends on the conditions under which it is used. The inference is that it functions best where advantage is taken of its insulating effect against low dough-room temperatures.

Sullivan and Richards (5) find a marked superiority in favor of the cabinet method. The points of superiority enumerated are: drier, more mellow doughs that machine better; 1 to 2% more absorption may be used in the dough stage; optimum mixing time is a few minutes less than with open-trough fermentation; a shorter sponge time may be employed; the bread has softer texture and thinner cell walls; and the cabinet provides a measure of temperature and humidity regulation.

The superiority of cabinet fermentation is attributed to the greater

concentration of carbon dioxide over the cabinet sponge during the period from the breaking of the sponge to the time of doughing. Effective results were obtained when the concentration of carbon dioxide during this period was not less than 20% and best results were experienced at much higher levels of carbon dioxide. It is suggested that the sponge is permeable to the gases above it and that since carbon dioxide is more soluble in water, the sponge is probably more permeable to carbon dioxide than to oxygen or nitrogen. Moreover, it is suggested that carbon dioxide exercises its effect specifically, upon either the protein or certain oxidizing enzyme systems, rather than upon anaerobiosis.

These studies were not available in 1944 when it was decided to evaluate cabinet fermentation on a commercial scale under actual shop conditions. To accomplish this, a series of qualitative tests were planned wherein, over a period of time, the doughs and bread resulting from open-trough and cabinet sponges were compared to controls representing the normal shop practice. In the test doughs, sponge time was checked at 30 minute increments from 4 hours 30 minutes to 3 hours. In a second series of tests, in an attempt to attain some objectivity, the rise of the sponges was measured. The carbon dioxide content of the atmosphere within the cabinet was measured at three different levels and above the sponge as fermentation progressed. An endeavor was made to note differences in fermentation characteristics through an adaptation of the gassing power test, and crumb compression measurements were made on the bread as well as observations on bread quality and characteristics. In addition, observations were made on dough-room temperature, cabinet temperature, and sponge temperature increase.

Materials and Methods

The work to be reported herein made use of a three-trough capacity cabinet constructed of plywood and illustrated in Fig. 1. Since the study extended over a considerable period of time, no attempt was made to work with a single lot of flour. However, unless otherwise specified, the flours used were milled from hard winter wheat and conformed to the following specifications:

% Protein (14% moisture basis).....10.5% to 11.0%,
% Ash (14% moisture basis).....0.42% maximum,
Gassing power (6 hours).....400–450 mm. Hg.,

where gassing power is determined by the method described in *Cereal Laboratory Methods* (1).

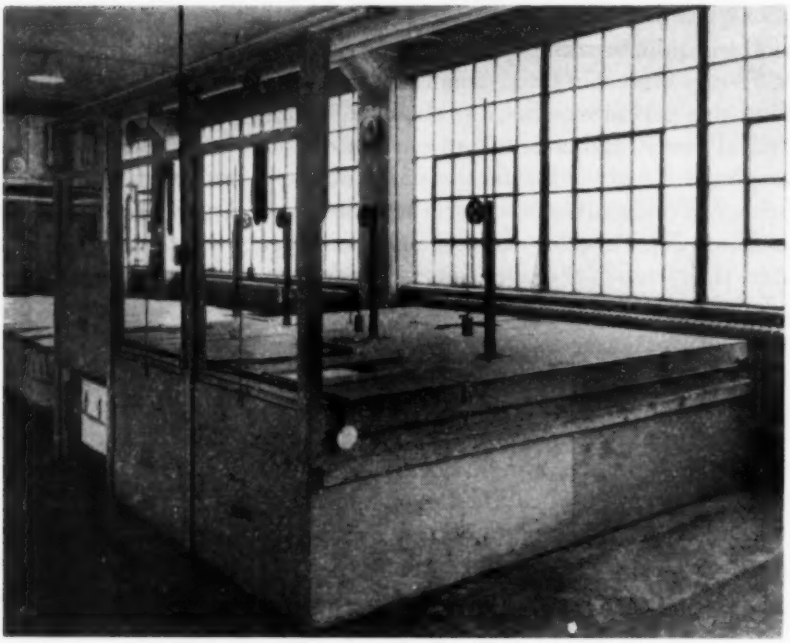

Fig. 1. Experimental cabinet installation.

A typical commercial formula making use of a 60% sponge was employed. The normal sponge time employed in the shop was 5 hours. The sponges were mixed 5–6 minutes and were set at 78°–79°F. (25.5°–26.1°C.). The doughs were mixed in accordance with the requirement of the flour (10 to 14 minutes), were set at 78°–80°F. (25.5°–26.7°C.), and were subjected to 20 minutes' floor time. An attempt was made to maintain the dough room at 80°F. (26.7°C.), although experience demonstrated that it varied between 79° and 85°F. (26.1° and 29.4°C.). The doughs were handled over the usual make-up machinery with a minimum of intermediate proof. Conditions maintained in the steam-box were 98°–100°F. (36.7°–37.8°C.), with relative humidity at 90% or higher. The bread was baked with a little steam in the oven and in accordance with commercial practices as to temperature and time. A 22-ounce plain-top loaf was made throughout this study.

The work was carried out in two stages. The first consisted of a series of qualitative tests which provided a background of experience for designing the second series of tests in which an attempt was made to measure objectively some of the factors involved.

Qualitative Tests. The technique employed in the qualitative

tests was to set three cabinet sponges and two open-trough control sponges each day. In order to "condition" the cabinet, it was filled with three sponges which were not involved in the test and which were so scheduled that, as each one was ready for remixing, a test cabinet sponge took its place in the cabinet. For the first series, the sponge time for the cabinet sponges was arbitrarily established as 4 hours 30 minutes. One of the open-trough control sponges was given the same sponge time and the other was run on the regular shop schedule of 5 hours. Then, 4-hour sponges in the cabinet were compared with a 4-hour sponge and a 5-hour sponge on the floor. The technique was repeated, each time reducing the sponge time of the cabinet sponges by 30 minutes and comparing them with controls in open-troughs, one at 5 hours and the other comparable in sponge time to the cabinet sponges. This process was continued until a sponge time of 3 hours had been tested.

Objective Tests. The second phase of the study consisted of three tests making use of hard winter wheat flours conforming to the specifications already described. The tests were so spaced as to include hard winter wheat flours from the end of the 1943 crop, the forepart of the 1944 crop, and the first third of the main body of the 1944 crop.

In these tests the rise of the sponges was measured objectively. The carbon dioxide content of the atmosphere within the cabinet was measured at three different levels as well as over the sponges as fermentation progressed. An attempt was made to note differences in fermentation characteristics through an adaptation of the gassing power test, and crumb compression measurements were made on the bread.

The device which was improvised to measure the rise of the sponges is illustrated in Fig. 2. Essentially it consists of a 12-inch by 12-inch metal plate *A* attached to a square rod *B* so mounted and guided as to permit only vertical movement. The system is so counterbalanced that the plate can follow any recession of the sponge, but cannot exert sufficient pressure to cause it to settle into the sponge. An arbitrarily chosen scale *C* is mounted adjacent to rod *B*. On the rod is an adjustable pointer *D* to facilitate the measurement of sponge movement. When a sponge is set, the "riseometer" is mounted over the sponge in the approximate center of the trough. The plate is lowered until it is just in contact with the sponge and then the adjustable pointer is set at "3." As fermentation progresses, the operator notes the scale readings at definite intervals of time. From these data can be constructed total-rise graphs or rate-of-rise graphs which are not dissimilar in their general contours to the analogous graphs that are

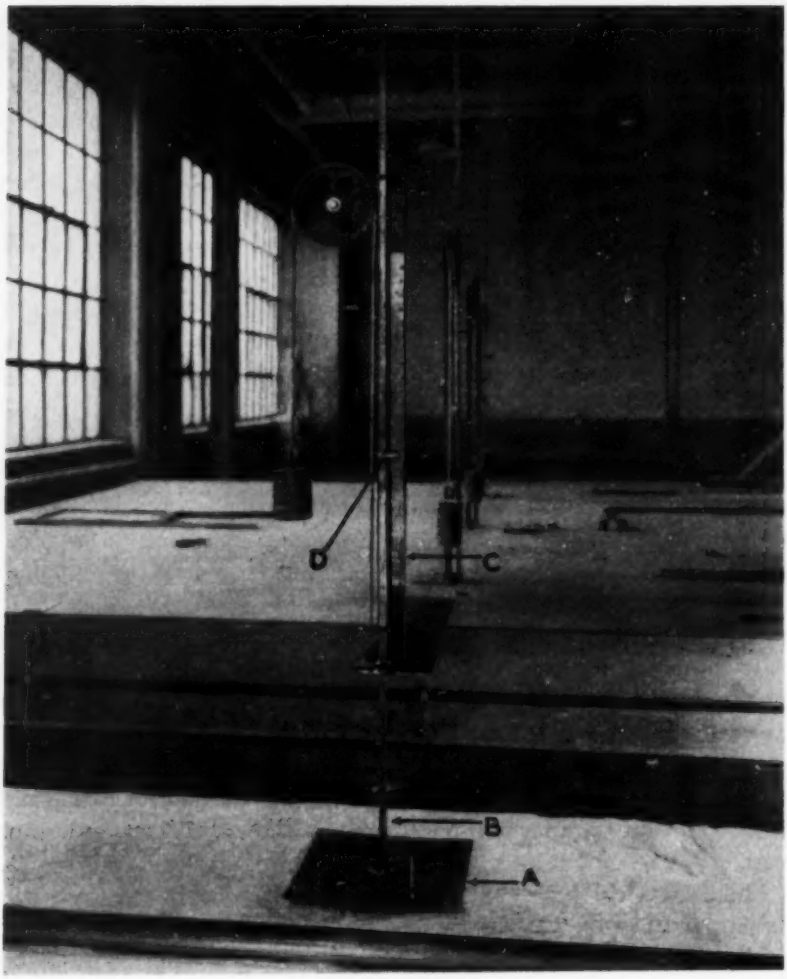

Fig. 2. Riseometer.

obtained from the results of gassing power tests, at least up to the time the sponge reaches its maximum height.

An Orsat apparatus was employed to determine the concentration of carbon dioxide at three levels in the cabinet and at a point about one inch above the surfaces of the cabinet sponges. The three levels in the cabinet corresponded, respectively, to heights 6 inches above the floor, about one-half that of the trough, and the top edge of the trough. Determinations were made as frequently as facilities and personnel available permitted over the period of time beginning with the entry of the first test sponge into, and the exit of the last test sponge from,

the cabinet which was not compartmentalized. No attempt was made to accumulate similar data for the dough room or for the space over the sponges therein, because the primary interest was in the cabinet conditions.

As another means of gaining insight into the progress of fermentation in cabinet sponges as compared with sponges fermented in open troughs in the dough room, aliquots of sponge were taken after fermentation had progressed 1, 2, 3, and 4 hours, were transferred to pressuremeters, and were then fermented at 30°C. for 6 hours. The hourly pressure readings were plotted and the contours and slopes of the various graphs, grouped according to the stage of sponge time, were compared.

The bread resulting from these tests was measured for crumb compressibility over a holding period of 5 days. The machine utilized was made to the specifications of Combs (2) and compressibility was expressed as the weight of mercury required to compress a sample one inch thick, an amount corresponding to 0.35 inch.

Results and Discussion

Qualitative Tests. One of the first things observed was that the cabinet sponges differed in appearance from the sponges on the floor. They did not crust and the top surface remained soft and moist. The gas cells were smaller and more uniformly distributed. The sponges from the cabinet felt drier when manipulated. However, the resultant bread was quite uniform in quality whether it was made from a cabinet sponge, an open-trough sponge of comparable time, or a 5-hour open-trough sponge. Nor did the make-up personnel note any differences in machining properties between any of the test doughs.

As a result of the observation on the appearance and the "feel" of the cabinet sponges, the test was repeated with one factor changed: the doughs from the cabinet sponges were given an increment of 1% extra absorption. All dough mixing times were kept the same. Again no advantages in bread quality could be noted, and it was concluded that the cabinet-method doughs do not possess extra absorption capacity. These doughs, however, machined satisfactorily.

Until a 3-hour sponge time was worked with, it was found that all of the bread produced in these tests was of comparable quality. The 3-hour sponges from the cabinet produced bread no better than that produced by the 3-hour sponge fermented in an open trough. Compared with the 5-hour sponge fermented in an open trough, the 3-hour sponges (from cabinet or open trough) produced bread slightly inferior in quality—not as good dough-to-dough or day-to-day uniformity.

As the result of this first set of experiments it was tentatively concluded that cabinet fermentation is not effective in reducing sponge time, increasing the absorption of the dough, improving the machining properties of the dough, or raising the quality of the bread. It was felt, however, that the cabinet does minimize the effect of temperature fluctuation in the dough room and effectively and economically provides adequate humidity to prevent sponges from crusting.

Objective Tests. In general, the practices and conditions already described were followed except that a sponge time of 4 hours 30 minutes was used in all tests. After the cabinet had been "conditioned," two normal-size sponges and one half-size sponge were placed in it. These were compared with a normal size and a half-size sponge fermented outside the cabinet in open troughs. Since the quantity of sponge (570 pounds) normally used in the shop where this work was done completely fills the trough at full rise, thus permitting any "blanket" of carbon dioxide to cascade off, the half-size sponge was made in the cabinet to insure that a high concentration of carbon dioxide would be retained over it.

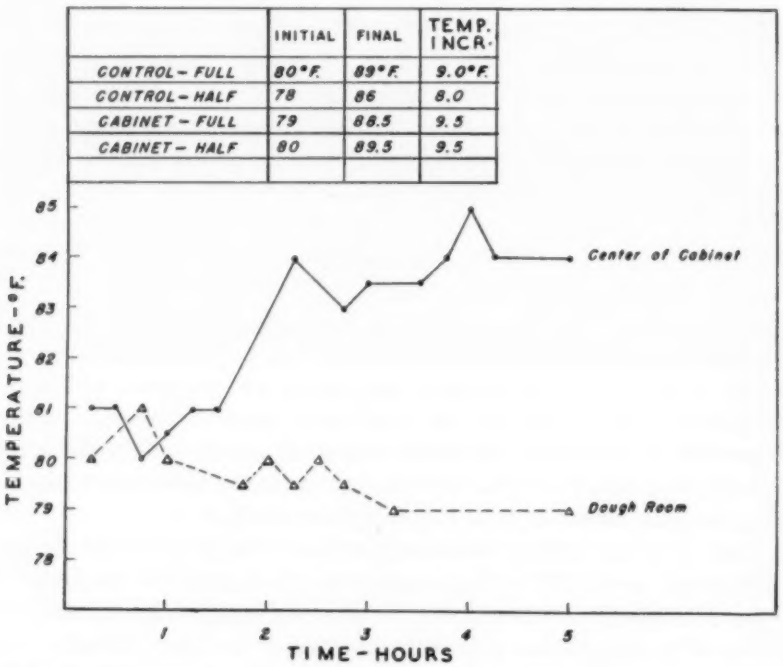

Fig. 3. Progression of temperature in the cabinet and in the dough room over the test period with lights operating in the cabinet, along with the temperature characteristics of the test sponges and the temperature increase of the sponges. Test 1, utilizing a hard winter wheat flour (1943 crop), half- and full-size sponges in room and cabinet, and a sponge period of 4 hours 30 minutes.

Tests 1 and 2 were identical except that the flour used, while conforming to the specifications already given, came from different shipments and different mills. In the cabinet were two full-size and one half-size sponges, while in the dough room in open troughs were a full-size and a half-size sponge. All were fermented 4 hours 30 minutes. Unless otherwise specified, all other conditions fell within the ranges already described.

Test 3 made use of a third lot of hard winter wheat flour, as already specified, and a spring wheat flour, generously furnished by Dr. Betty Sullivan of the Russell-Miller Milling Company. It analyzed 0.40% ash and 11.5% protein. Since the quantity of this flour was limited, it was not possible to mix it any more than was the hard winter wheat flour; and the absorption applied was on the conservative side of the flour's requirement. In the "conditioned" cabinet were fermented a full-size hard winter wheat flour sponge, a full-size spring wheat flour sponge, and a half-size spring wheat flour sponge. In the dough room were fermented in open troughs a full-size hard winter wheat flour sponge, a full-size spring wheat flour sponge, and, in a covered trough,

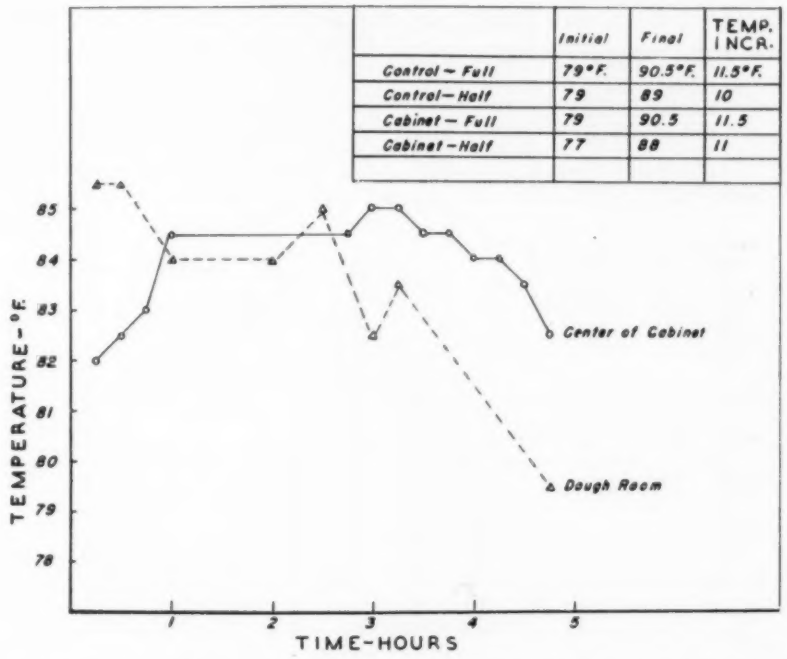

Fig. 4. Progression of temperature in the cabinet and in the dough room over the test period without lights operating in the cabinet, along with the temperature characteristics of the test sponges and the temperature increase of the sponges. Test 2, utilizing a hard winter wheat flour (early 1944 crop), half- and full-size sponges in room and cabinet, and a sponge period of 4 hours 30 minutes.

a full-size spring wheat flour sponge. All sponge times were 4 hours 30 minutes and, unless otherwise specified, the shop conditions were as previously noted.

Figs. 3, 4, and 5 record the march of temperature over the three test periods in the cabinet and in the dough room itself. In addition, they show the temperature increase of the sponges.

Fig. 3 covers test 1, during which the lights in the cabinet were permitted to operate. As a consequence, the cabinet temperature built up, despite a tendency for the dough-room temperature to diminish. This fact emphasizes the insulating effect of the cabinet and suggests that, in emergencies, the burning of a few incandescent lamps would be effective in raising the cabinet temperature. The increases in temperature of the corresponding sponges in open troughs and in the cabinet are little different and can be accounted for readily by environmental temperatures and relative exposure of the sponges thereto.

In test 2 (Fig. 4) the lights were not used. In general, the cabinet tended to follow the temperature trend of the room; a lag in its re-

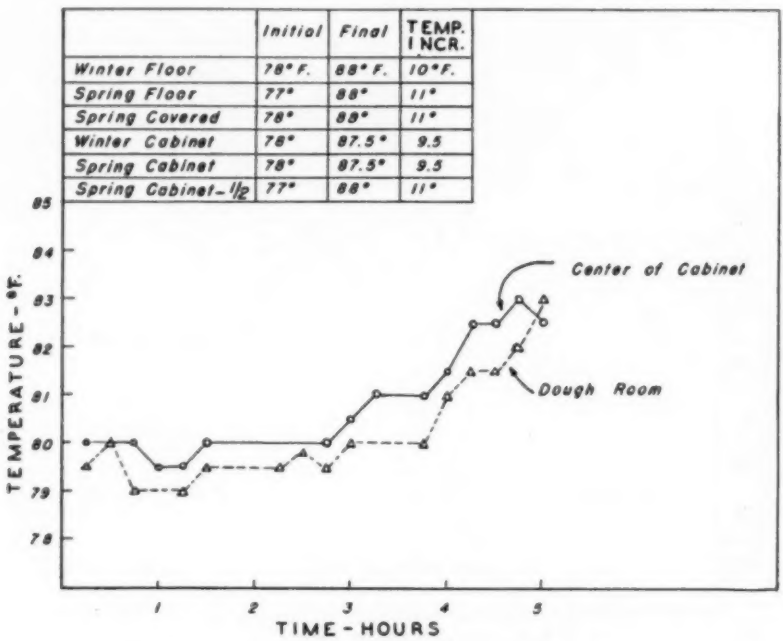

Fig. 5. Progression of temperature in the cabinet and in the dough room over the test period without lights operating in the cabinet, along with the temperature characteristics of the test sponges and the temperature increase of the sponges. Test 3, utilizing a hard winter wheat flour (1944 crop) and a spring wheat flour, with sponges in room and cabinet as described, and a sponge period of 4 hours 30 minutes.

sponse is apparent. The full sponges, whether in the cabinet or in an open trough, experienced the same temperature increase. The temperature increases in the half-size sponges fermented in the cabinet and in an open trough are very similar. Any differences can be accounted for by the difference in the environmental temperatures in which they resided.

Fig. 5 illustrates the degree of parallelism in temperature between the room and the cabinet. There is a general tendency for the cabinet temperature to be slightly higher than that of the room. The sponges made from hard winter wheat flour registered no practical difference in temperature increase. Among the full sponges made from spring wheat flour, that in the open trough and that in the covered trough developed the same temperature increase. The behavior of the full-size spring wheat flour sponge in the cabinet is difficult to explain, but it may illustrate the limitations under which sponge temperatures are measured under shop conditions. The temperature increase for the half-size spring wheat flour sponge is somewhat more in line with the general picture.

Figs. 6, 7, and 8 depict the concentrations of carbon dioxide in the cabinet over the test period and at the three levels checked. Points *A*, *B*, and *C* indicate the removal of the sponges used to "condition"

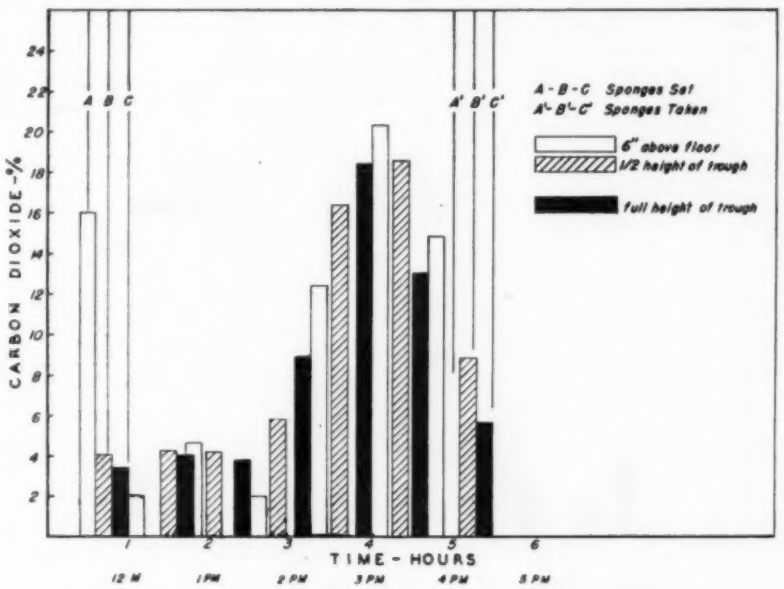

Fig. 6. Concentration of carbon dioxide in the cabinet at three levels at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

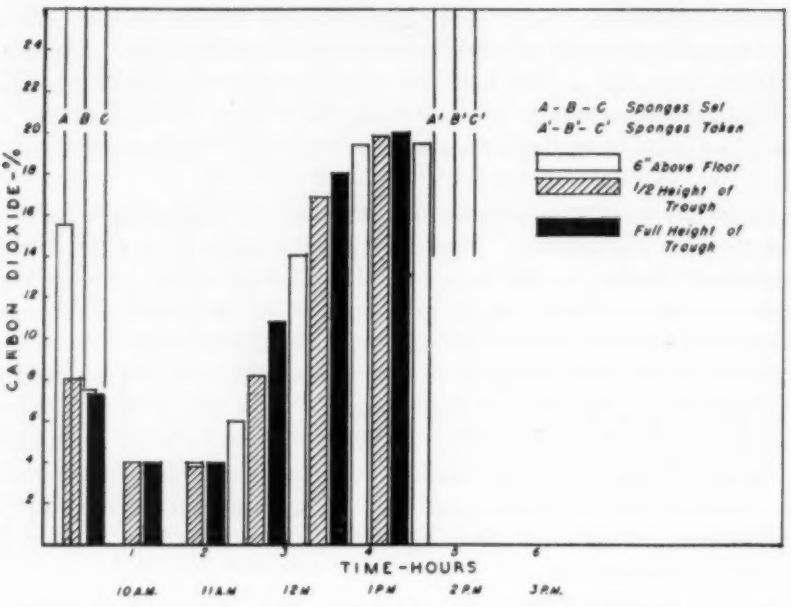

Fig. 7. Concentration of carbon dioxide in the cabinet at three levels at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

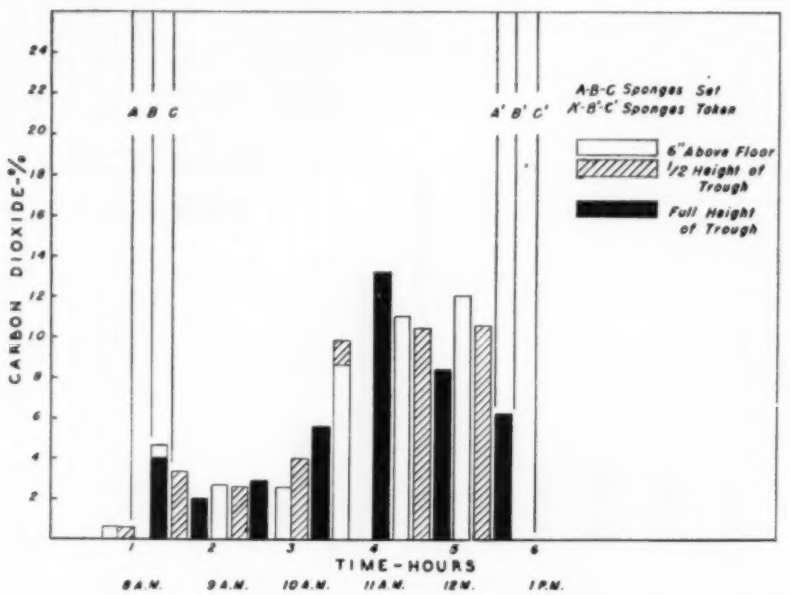

Fig. 8. Concentration of carbon dioxide in the cabinet at three levels at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 3, utilizing a hard winter wheat flour (1944 crop), a spring wheat flour, and a sponge period of 4 hours 30 minutes.

the cabinet and the introduction of the test sponges. Points A^1, B^1, and C^1 are the times at which the test sponges were removed, thus progressively emptying the cabinet.

Figs. 6 and 7 have several points of similarity and suggest that the carbon dioxide concentration built up during the "conditioning" period is rather effectively diluted by the traffic into and out of the cabinet. Then the carbon dioxide concentration builds up gradually to about 18 to 20% relatively late in the sponge time. There is some evidence that cascading from the full sponges is experienced and that stratification develops, although this is not pronounced. Finally, traffic causes the carbon dioxide concentration in the cabinet to diminish markedly.

Fig. 8 showing the results for two sponges made from spring wheat flour and one sponge made from hard winter wheat flour, reflects approximately the same sequence of events, except that the maximum concentration of carbon dioxide is only 12 to 14%. Whether this is characteristic for the spring wheat flour used or the result of an unknown experimental error could not be determined.

The concentration of carbon dioxide approximately one inch above

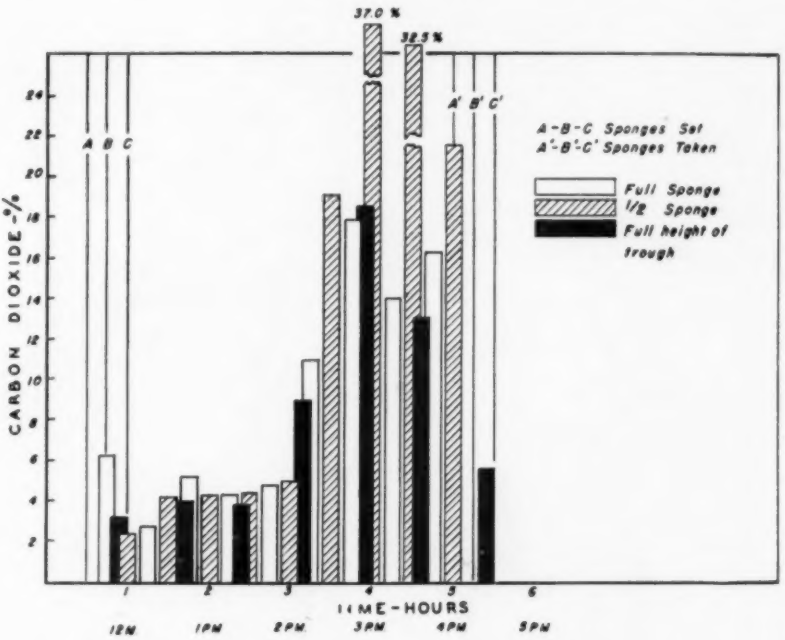

Fig. 9. Concentration of carbon dioxide in atmosphere within cabinet, one inch above the test cabinet sponges, and at a level corresponding to the height of the trough at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

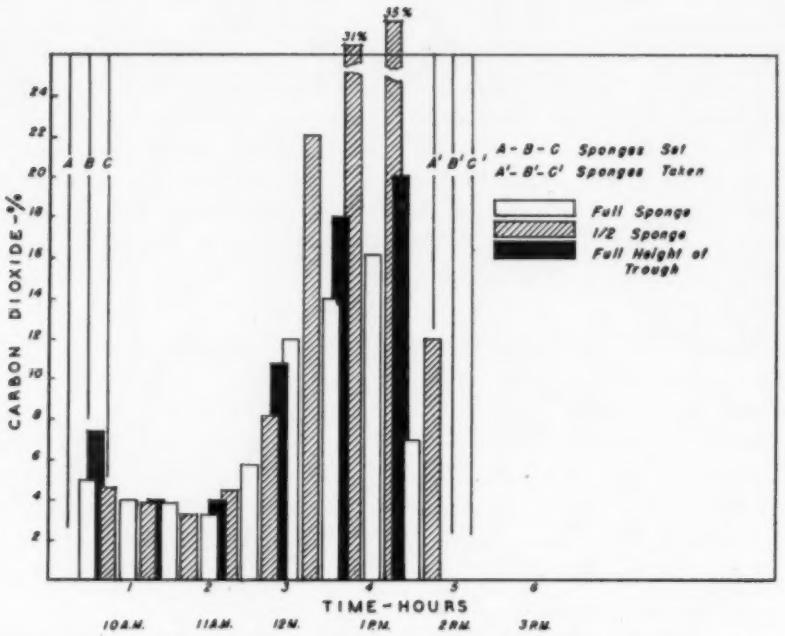

Fig. 10. Concentration of carbon dioxide in atmosphere within cabinet, one inch above the test cabinet sponges, and at a level corresponding to the height of the trough at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

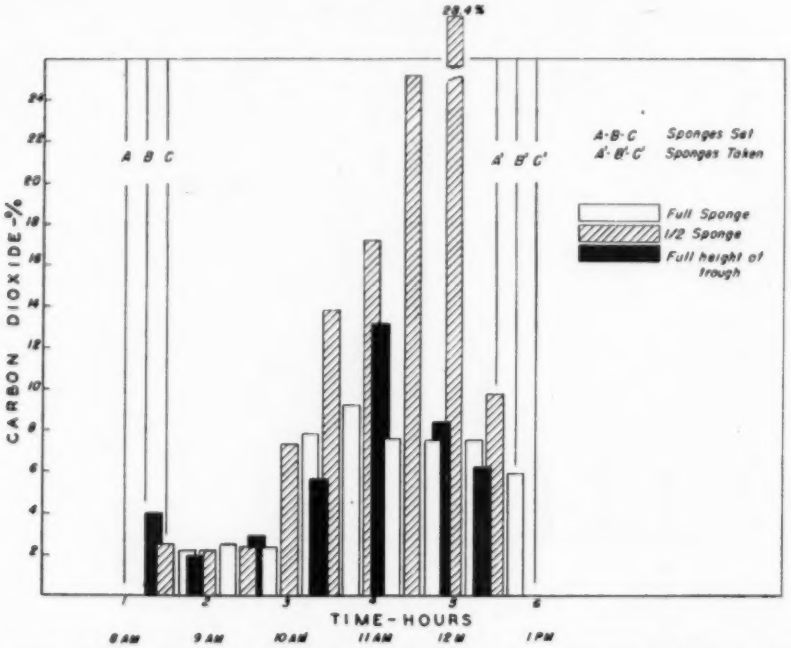

Fig. 11. Concentration of carbon dioxide in atmosphere within cabinet, one inch above the test cabinet sponges, and at a level corresponding to the height of the trough at intervals over the test period, in relation to introduction and withdrawal of the test sponges. Test 3, utilizing a spring wheat flour and a sponge period of 4 hours 30 minutes.

the surface of the sponge is shown in Figs. 9, 10, and 11. For example, Fig. 9 brings out that the full-size sponge and the half-size sponge each develop a maximum concentration of carbon dioxide within about 3¼ hours; but that the level attained over the full sponge was almost 18% as compared with 37% over the half sponge.

In test 2 illustrated by Fig. 10, the maxima appear within approximately 3¾ hours. That over the full sponge is slightly more than 16% compared with 35% over the half sponge.

In test 3, which involved the use of spring wheat flour, the progression of carbon dioxide concentration over the cabinet sponges is depicted in Fig. 11. The maximum concentration over the full sponge would seem to come within about 2¼ hours, which is erratic when compared with the appearance of the maximum concentration

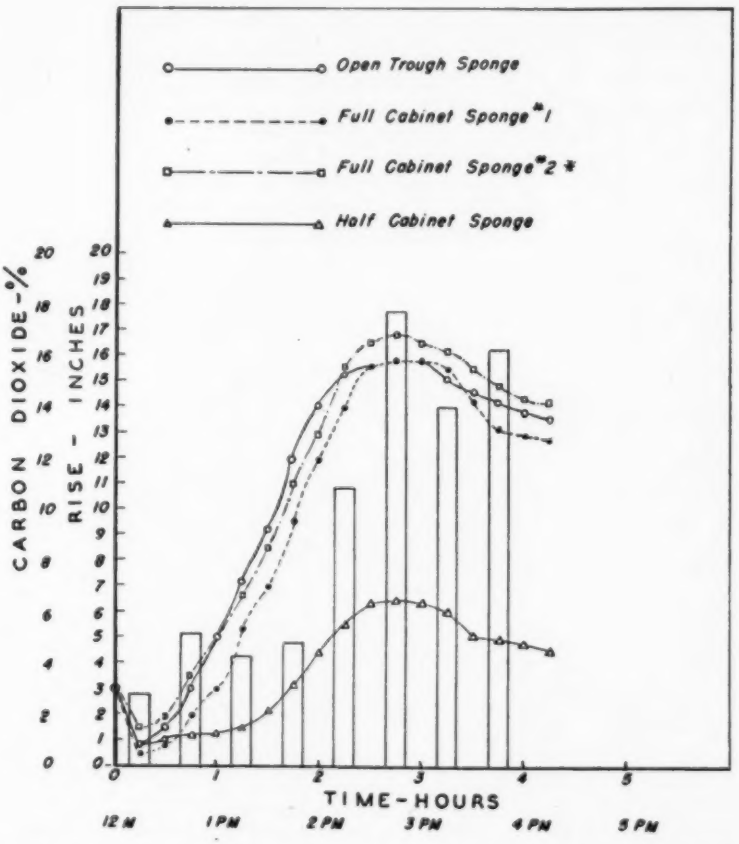

Fig. 12. Progression of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 2. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

over the half sponge at about 3¾ hours. The only plausible explanation for this is that the appearance of the true maximum for the full sponge is obscured by the cascading of the carbon dioxide off the surface of this sponge, which stood high in the trough. Whatever the cause, the maximum carbon dioxide concentration over the full sponge was slightly in excess of 9% and over the half sponge, a little more than 28%. Both of these values are lower than those encountered in the two previous trials in which hard winter wheat flour was used.

The rise of the sponges is plotted in Figs. 12, 13, and 14, utilizing the "riseometer" already described. Fig. 12 shows graphs obtained for a full sponge in an open trough, two full sponges in the cabinet, and a half-size sponge in the cabinet. The two full cabinet sponges provide some idea of the degree of replication that can be attained

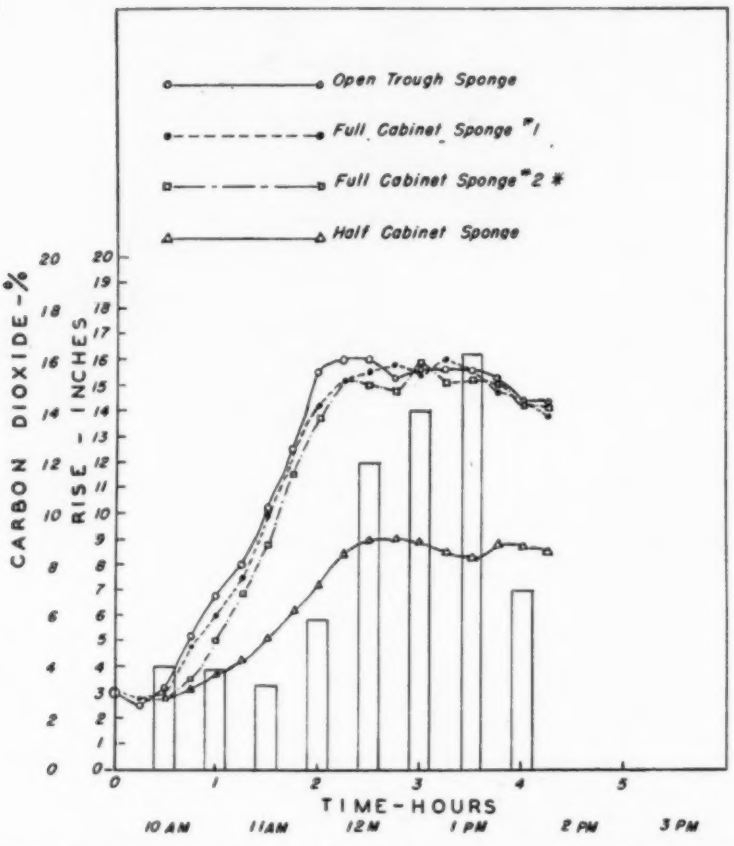

Fig. 13. Progression of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 2. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

with this device and hence assist in interpreting the results. With such provision as a guide, it is apparent that in test 1 the sponge fermented in the open trough did not behave any differently from the two cabinet sponges. The graph for the half-size cabinet sponge is given to show the parallelism existing between it and the full-size sponges in the cabinet, although it had over it, for a measurable period of time, a significantly higher concentration of carbon dioxide. The bar graph shows the progression of carbon dioxide concentration over the cabinet sponge designated number 2.

The results of test 2 are shown in Fig. 13. This experiment was conducted with a hard winter wheat flour differing in source and crop year from that used in test 1. It is evident that its fermentation char-

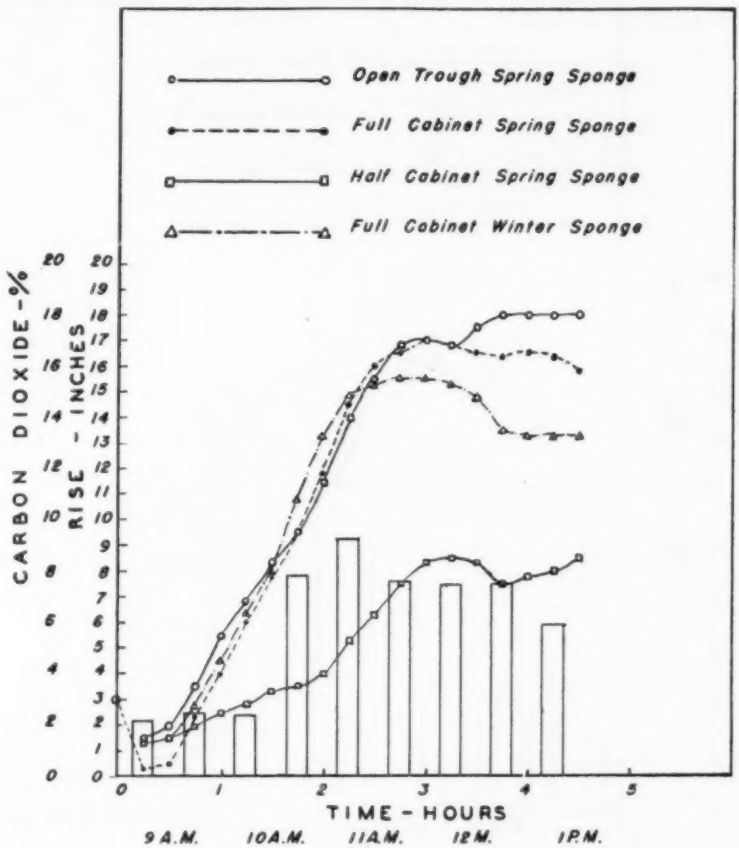

Fig. 14. Progression of rise of test cabinet sponges made with a spring wheat flour in comparison with a comparable open-trough sponge and a test cabinet sponge made with a hard winter wheat flour (1944 crop) and in relation to concentration of carbon dioxide, one inch above the full cabinet sponge made with spring wheat flour. Test 3, utilizing a sponge period of 4 hours 30 minutes.

acteristics vary from those of the flour used in test 1, yet the graphs for the full sponge in the open trough and the two full cabinet sponges practically coincide. Again there is marked parallelism between the half sponge in the cabinet and the full cabinet sponges which developed lower concentrations of carbon dioxide over their surfaces. Also, the bar graph illustrates the progression of carbon dioxide over cabinet sponge 2 in relation to its expansion in the trough.

The results of test 3 are illustrated in Fig. 14. It contains "riseometer" graphs for spring wheat flour sponges, including a full sponge in an open trough, a full sponge in the cabinet, and a half sponge in the cabinet. Besides, there is a graph for a full cabinet sponge made from a hard winter wheat flour.

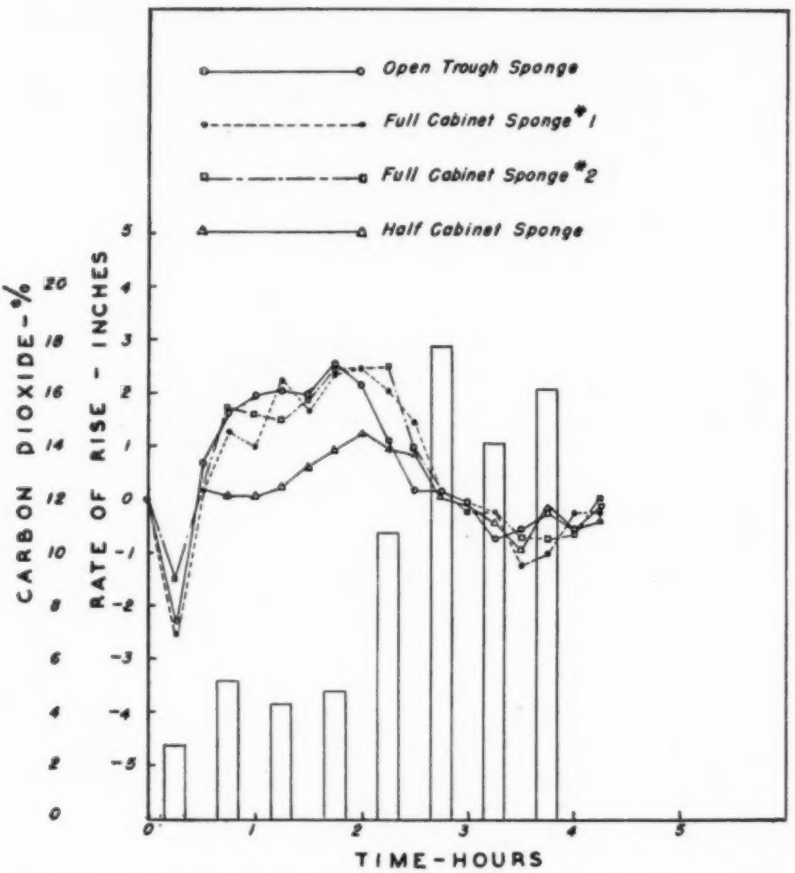

Fig. 15. Rate of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 2. Test 1, utilizing a hard winter wheat flour (1943 crop) and a sponge period of 4 hours 30 minutes.

First of all, there is noted a characteristic difference between the hard winter wheat flour sponge and the two full-size spring wheat flour sponges, which is not contrary to expectations. There is great similarity between the open-trough and cabinet sponges utilizing spring wheat flour. The divergence apparent in the latter stage of the sponge period can be attributed to the crusting which was encountered on the sponge in the open trough. The graph for the half cabinet sponge reasonably parallels the comparable full-size sponge. The latter portion of the curve is in all probability influenced by the confinement of the sponge within the trough, whereas the full cabinet sponge to some extent overflowed its trough. The bar graph portrays

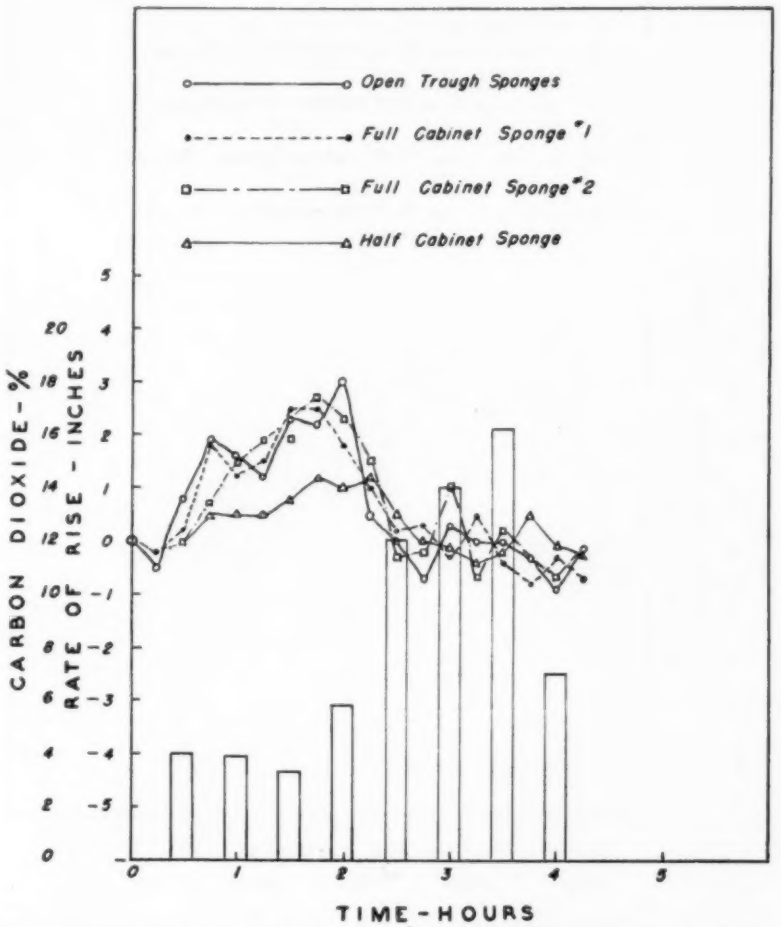

Fig. 16. Rate of rise of test cabinet sponges in comparison with a comparable open-trough sponge and in relation to concentration of carbon dioxide, one inch above cabinet sponge number 2. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

the march of carbon dioxide over the full cabinet sponge made with spring wheat flour. When related to its "riseometer" curve, and having in mind the comparable relationships to be observed in Figs. 12 and 13, there is reason to believe that the appearance of the maximum is premature and that the true maximum concentration of carbon dioxide was not realized, probably because the carbon dioxide cascaded from the sponge's surface.

Figs. 15, 16, and 17 show the data from the "riseometer" readings as rate curves and in this form depict a high degree of similarity between open-trough sponges and cabinet sponges in the progress of fermentation.

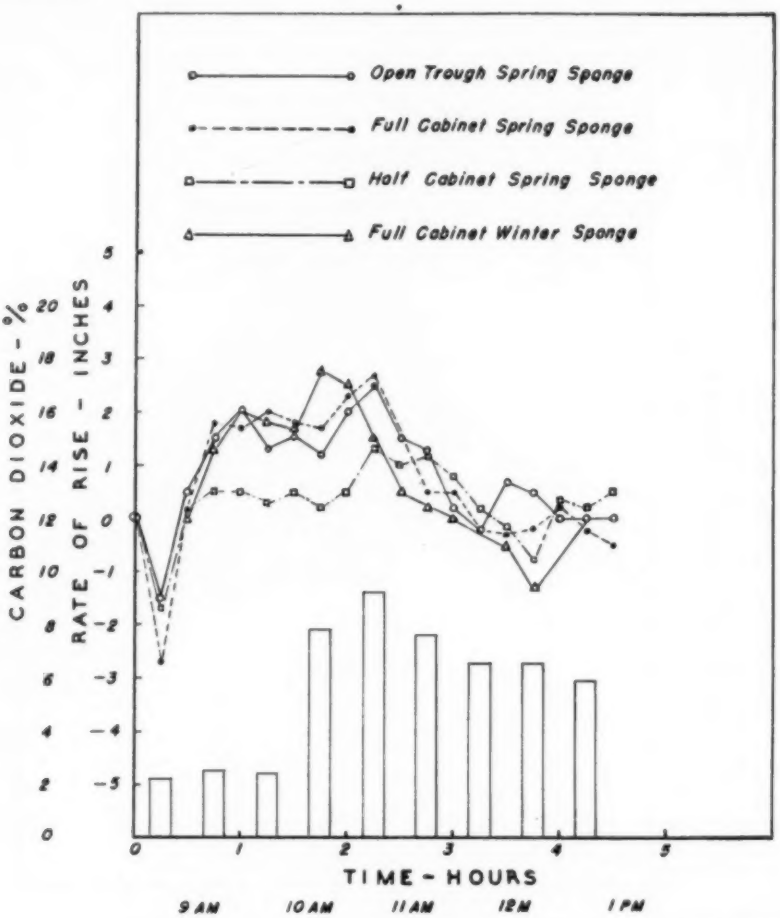

Fig. 17. Rate of rise of test cabinet sponges made with a spring wheat flour in comparison with a comparable open-trough sponge and a test cabinet sponge made with a hard winter wheat flour (1944 crop) and in relation to concentration of carbon dioxide, one inch above the full cabinet sponge made with spring wheat flour. Test 3, utilizing a sponge period of 4 hours 30 minutes.

The results from the adaptation of the gassing power tests are given in Figs. 18 and 19, and cover only tests 2 and 3, since its use was not conceived until test 1 had been carried out. Having in mind the conditions under which the tests were made in the shop, as well as the recognized limitations residing in pressuremeter tests with respect to concordance in absolute terms, it is concluded that these results portray no practical difference in fermentation between sponges

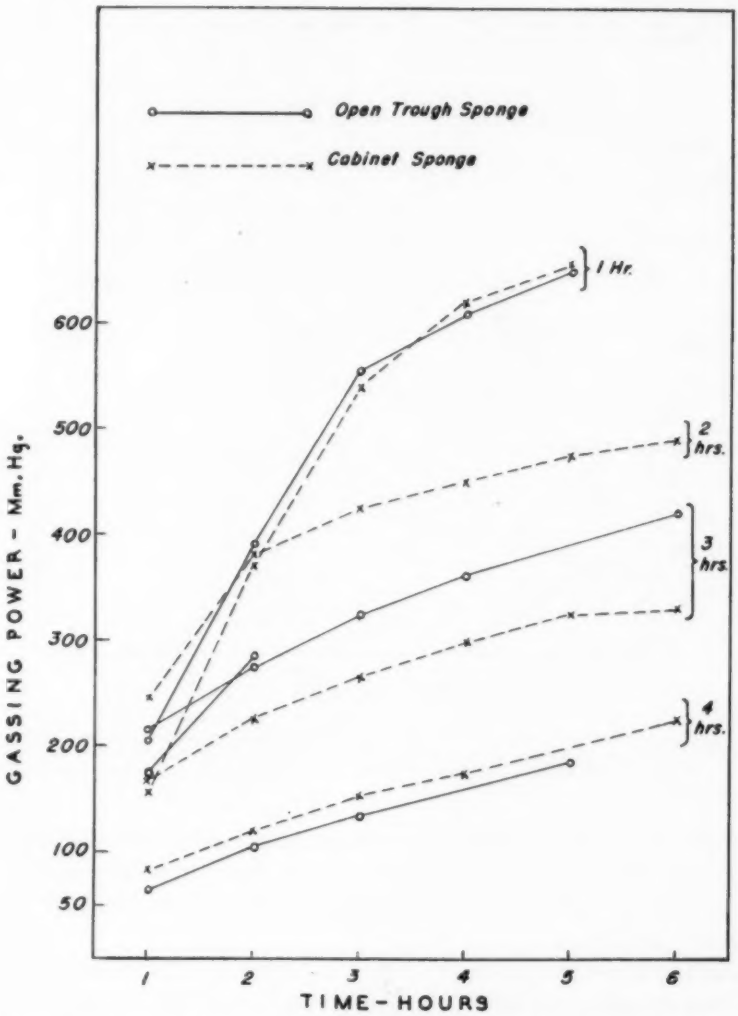

Fig. 18. Comparison of gassing power of open-trough and cabinet sponges, utilizing aliquots of sponge taken after 1, 2, 3, and 4 hours of fermentation and fermented for 6 hours in a pressuremeter at 30°C. Test 2, utilizing a hard winter wheat flour (early 1944 crop) and a sponge period of 4 hours 30 minutes.

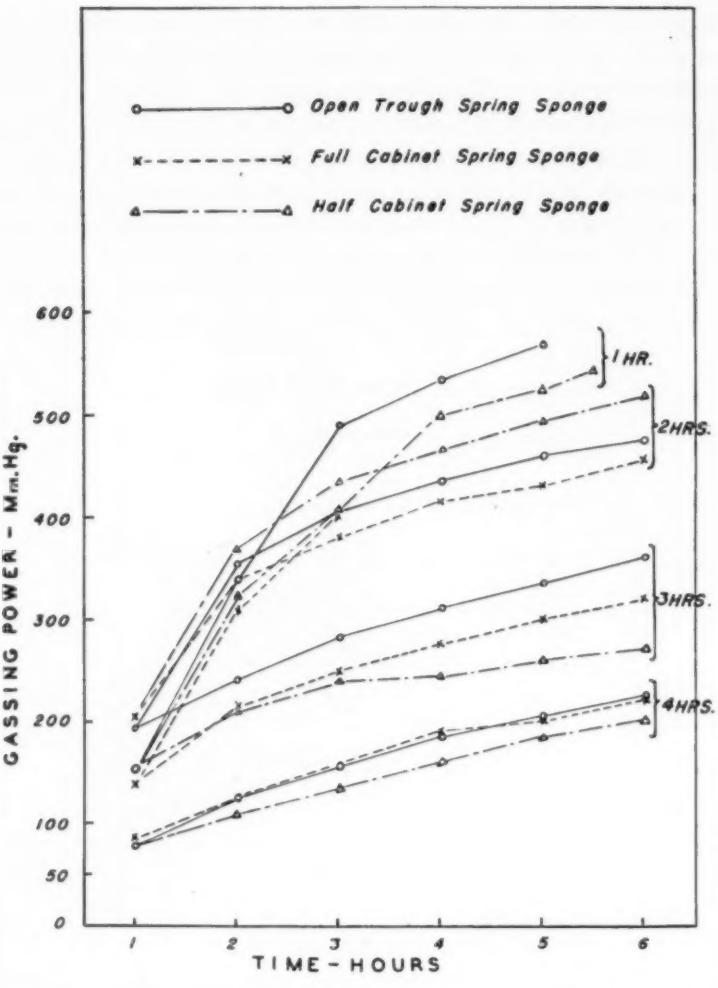

Fig. 19. Comparison of gassing power of an open-trough sponge with a full and half cabinet sponge, utilizing aliquots of sponge taken after 1, 2, 3, and 4 hours of fermentation and fermented for 6 hours in a pressuremeter at 30°C. Test 3, utilizing a spring wheat flour and a sponge period of 4 hours 30 minutes.

fermented in open troughs and sponges fermented in the cabinet. The sponge aliquots of equal fermentation time definitely group together, while the slopes of their pressuremeter curves reflect a high degree of parallelism.

As in the series of qualitative tests, no difference in the machinability of the doughs was noted by the personnel at the make-up units. Even the half-size sponges fermented in the cabinet, which had a significantly greater concentration of carbon dioxide over them, showed no differences.

An adequate number of sample loaves were taken from each test dough by the shop foreman and, on the day following the test, were examined by a panel of four. After careful examination, the consensus was that the quality and characteristics of the bread were comparable, regardless of whether the sponges were fermented in open troughs or in the cabinet, or whether the bread was made from half-size sponges fermented by the two methods.

	Open	Cab. Full	½ Open	½ Cab.
1 Day	5.9	6.0	5.7	5.8
2 Day	9.4	9.2	8.7	8.8
3 Day	11.8	12.2	11.8	11.8
5 Day	16.2	16.1	15.8	16.3

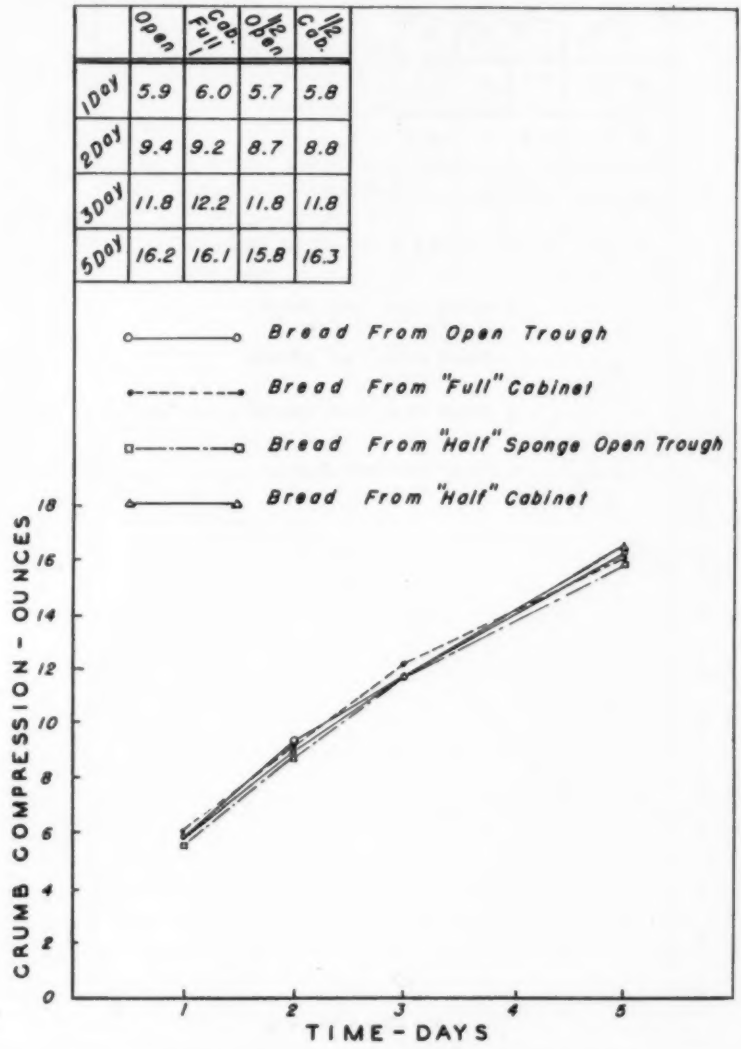

Fig. 20. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 1, utilizing bread made from hard winter wheat flour (1943 crop).

The results of crumb-compression measurements on the bread resulting from the tests are shown in Figs. 20, 21, 22, and 23. Measurements were made 1, 2, 3, and 5 days after baking. The resultant staling curves for test 1 are given in Fig. 20. They coincide so closely as to leave little room to attribute any superiority in softness or keeping quality to the bread made from cabinet sponges. Fig. 21 shows that comparable results were obtained in test 2.

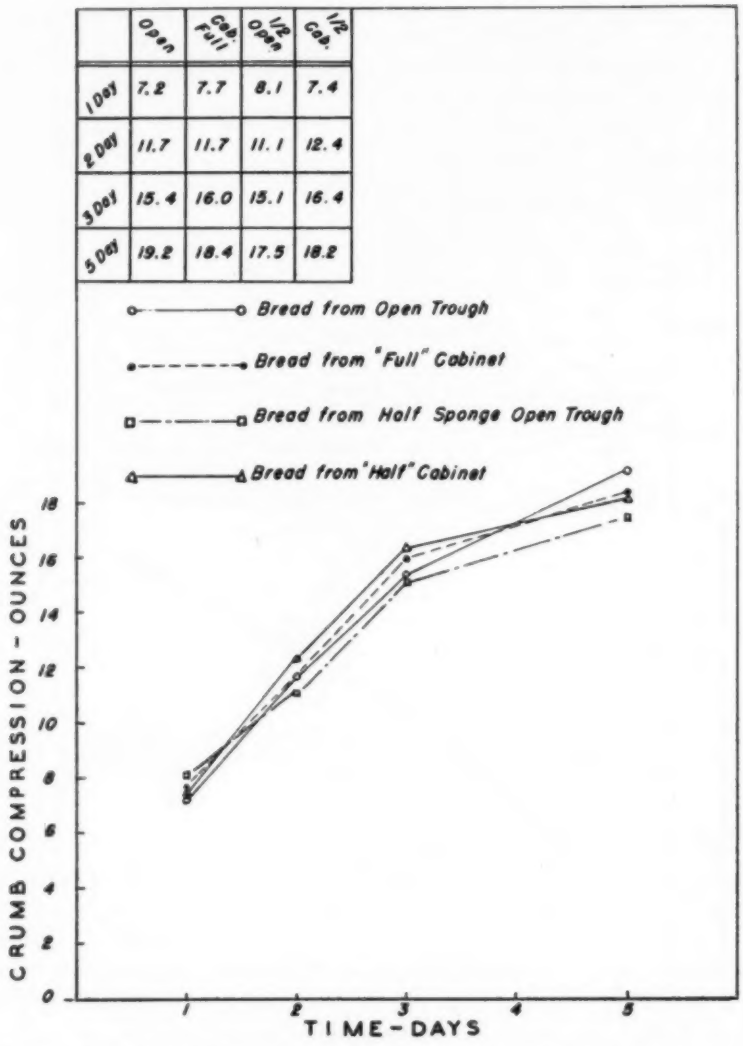

Fig. 21. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 2, utilizing bread made from hard winter wheat flour (early 1944 crop).

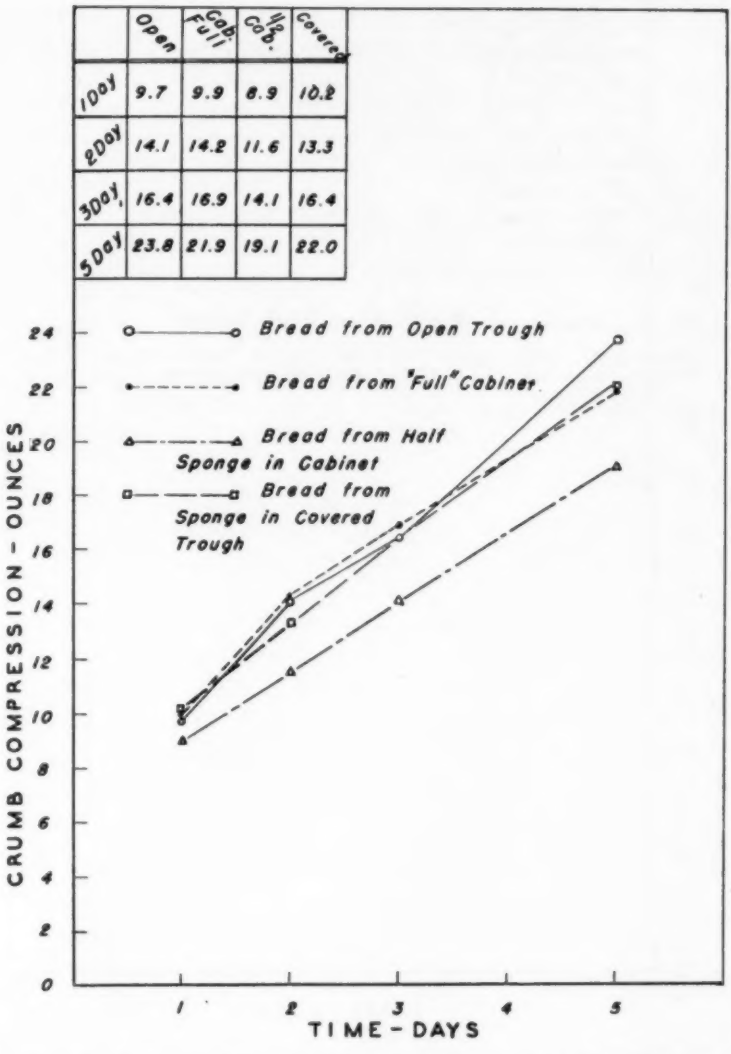

Fig. 22. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 3, utilizing bread made from spring wheat flour.

Fig. 22 illustrates the crumb compression results obtained in test 3 with the bread made from spring wheat flour. Only the bread resulting from the half-size sponge fermented in the cabinet shows any advantage in softness. The samples from the other three sponges, open-trough sponge, covered-trough sponge, and full-size cabinet sponge, are quite comparable in softness.

Fig. 23 also applies to test 3. It contains the crumb compression

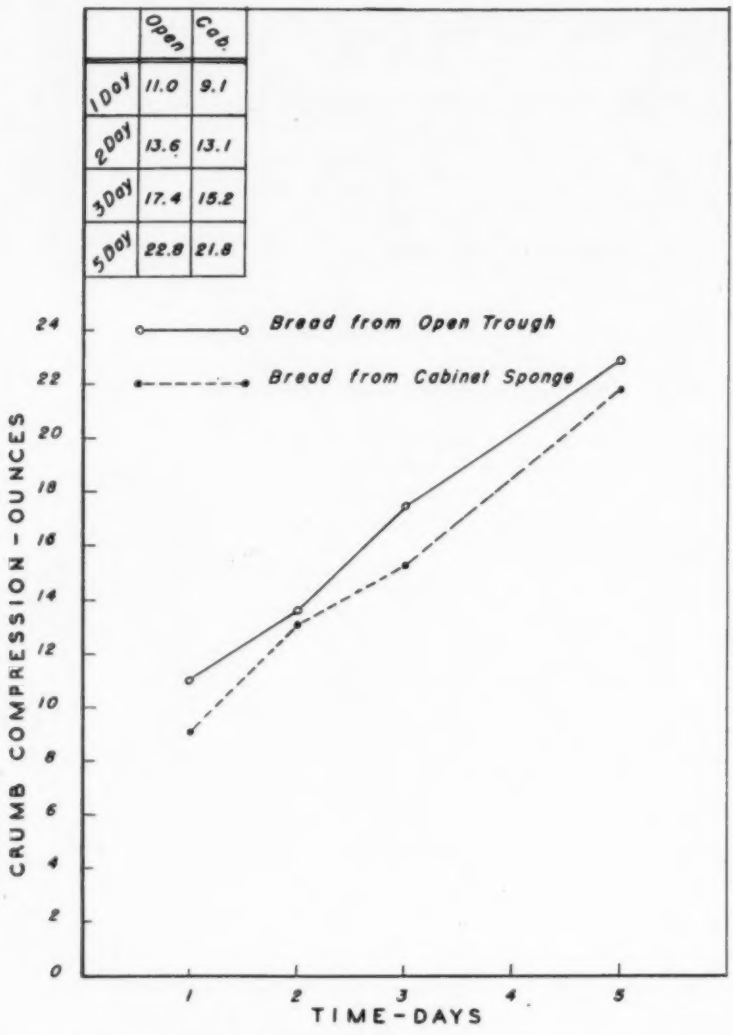

Fig. 23. Crumb compression measurements on bread from comparable open-trough and cabinet sponges made after 1, 2, 3, and 5 days holding at room temperature. Test 3, utilizing bread made from hard winter wheat flour (1944 crop).

results obtained with the bread made from hard winter wheat flour. The bread resulting from the cabinet sponge appears to be slightly softer than that made from the open-trough sponge, but in the light of the authors' experience in the application of this test, the difference is just barely significant.

Under the conditions of this study, therefore, the cabinet is ineffective except to insulate the sponges against significant fluctuations in dough-room temperature. It usually maintains a slightly higher

temperature than the dough room, and automatically provides sufficient humidity to prevent crusting of the sponges.

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MALTASE ACTIVITY OF VARIOUS MOLD BRANS AS EVALUATED BY SELECTIVE FERMENTATION [1]

ROBERT L. GATES [2] and ERIC KNEEN [3]

ABSTRACT

A simplified, roughly quantitative procedure for the estimation of maltase activity was developed. The method involves the digestion of maltose by enzyme extracts under specified conditions and the evaluation of glucose produced by a differential fermentation procedure. A yeast having a very low maltose fermentation capacity in the first stages of fermentation was used, and carbon dioxide production in the first hour following an initial 30 minutes of fermentation was measured manometrically and related to glucose content.

When applied to the study of mold brans prepared by culturing various fungal isolates, the method indicated a wide range in maltase activities. Some mold brans of low amylase activity showed high maltase activity, while in others, high amylase activity was no guarantee of high maltase activity. However, within a group of closely related isolates, for example, the *Rhizopus-Mucor* or the *Aspergillus oryzae*, there was a positive correlation between amylase and maltase activities.

There is considerable evidence to support the contention that the principal product from the action of the amylases on starch is maltose (5, 6, 14, 16). Indicative of the general acceptance of this fact is the custom of reporting amylase activity in terms of grams of maltose produced. To hydrolyze maltose to the fundamental unit of starch structure, glucose, a third enzyme must be brought into play. This enzyme, maltase or alpha-glucosidase, is frequently absent from amylase-active materials.

The exact role or importance to be ascribed to maltase in the various processes that involve the degradation of starch is open to question. Leibowitz and Hestrin (8) have furnished an excellent review of the possible role of maltase in the fermentation of maltose. They insist that the enzyme is unnecessary for the action of bakers' yeast on maltose but concede that it may play a part in fermentation of maltose by brewers' yeast. Contradictory evidence of the superficiality of maltase in the fermentation picture has been submitted by Corman and Langlykke (3). These workers showed that the alcohol production potential of a fungal saccharifying agent for corn mashes

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was more closely correlated with a "gluconic enzyme system, as measured by maltose hydrolysis, than with fungal alpha-amylase."

Certainly the action of maltase is important to those interested in the production of a syrup with a controlled glucose/maltose ratio by the enzymic degradation of starchy materials. Schultz *et al.* (12) discuss the matter of glucose and maltose content of syrups and compare the results of analysis by chemical and selective fermentation methods. Schwimmer (14) suggested that maltase has an important role in the degradation of raw starches by the removal of the end products of amylase action and elimination of certain inhibitory effects. From a theoretical point of view the maltase activity of an enzyme-containing material should be measured in order to complete the characterization of the carbohydrase system.

The measurement of maltase activity can be a tedious procedure due to the difficulties involved in the determination of glucose in the presence of maltose (9, 10). Many have turned to the selective fermentation method of Somogyi (15) who found that alkaline conditions suppressed the fermentation of maltose but not that of glucose. A similar method has been developed by Schultz and Kirby (13) in which they employ certain specific organisms for the removal of individual sugars from mixtures of several carbohydrates. Several workers have reported the differences that exist in the rate of fermentation of glucose and maltose by a given yeast (2, 4). Maltose requires a definite induction period before active fermentation occurs.

It seemed possible that the principle of selective fermentation might be applied by utilizing the unusual properties of one of the commercial dehydrated yeasts. The purpose of this report is to present the development of a method of maltase assay using a commercial dehydrated yeast and the application of the method to the screening of some 43 carbohydrase systems.

Methods

Extraction of Mold Bran. The mold brans were extracted for 1 hour at 30°C. with distilled water in a ratio of 10 g. of bran to 100 ml. of water. The extracts were then filtered through paper and desired aliquots used.

Fermentation Measurement. The carbon dioxide produced by yeast fermentation was evaluated manometrically in the "pressuremeter" described by Sandstedt and Blish (11) using mercury manometers. The aluminum cups proved very adaptable because they provide a rapid heat transfer. The method was similar to that applied to the evaluation of amylase "saccharifying activity" by Kneen and

Beckord (7), whose suggested yeast nutrient, a modification of the Atkin, Schultz, and Frey (1) nutrient, was used.

Results and Discussion

Selective Fermentation by Commercial Yeasts. Three commercial yeasts were tested for fermentation of maltose and glucose. One of these, "A," was a compressed product and the other two, "B" and "C," [4] were dehydrated products. For the fermentations each pressuremeter cup contained, in addition to the nutrients, 600 mg. sugar and 0.5 g. yeast, the total liquid volume being 30 ml. In Table I are recorded in abbreviated form the data for the first part of the fermentations.

TABLE I

SELECTIVE FERMENTATION OF MALTOSE AND GLUCOSE BY THREE COMMERCIAL YEASTS

(Carbon dioxide production expressed as mercury pressure)

Yeast[1]	Glucose (600 mg.)			Maltose (540 mg.), glucose (60 mg.)			Maltose (600 mg.)			
	1 hr.	2 hr.	5 hr.	1 hr.	2 hr.	5 hr.	1 hr.	2 hr.	5 hr.	8 hr.
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
"A"	90	254	414	70	216	484	17	81	382	437
"B"	147	307	455	28	73	424	12	21	185	411
"C"	119	277	454	23	42	153	10	14	30	57

[1] "A," compressed; "B" and "C," dehydrated.

It is obvious from Table I that the customary "induction period" with maltose was even more pronounced for the two dehydrated yeasts used than for the compressed yeast. This was particularly true of the "Maca" yeast. The present as well as previous studies encouraged the belief that a simple, rapid, roughly quantitative evaluation of glucose in the presence of maltose could be achieved by the use of this particular yeast.

Considerable experimentation made it evident that if 1.5 g. of "Maca" yeast was used per pressuremeter cup and the 15-minute manometer reading subtracted from the 75-minute reading, the difference was roughly proportional to glucose content and not influenced by maltose concentration. This is shown by the data of Table II.

Further investigations were made to establish the most nearly quantitative conditions for the fermentation. A range of yeast con-

[4] The "C" dehydrated yeast was marketed as "Maca." Subsequent to the completion of much of the research we were informed by the Northwestern Yeast Company that this product has been withdrawn from the market. Without doubt other nonmaltose fermenting yeast strains would serve as efficiently.

centrations from 0.5 to 2.0 g. per cup was used and the carbon dioxide production between 15 and 75 minutes, between 30 and 90 minutes, between 45 and 105 minutes, and between 1 and 2 hours of fermentation was correlated with actual glucose present. This information supported the previous evidence that 1.5 g. yeast was most satisfactory.

The 1-hour period during the course of the fermentation in which carbon dioxide production was most closely related to glucose content was finally determined to be that between 30 and 90 minutes rather than between 15 and 75 minutes of fermentation. These data are

TABLE II

FERMENTATION OF GLUCOSE BY "MACA" YEAST IN THE ABSENCE AND PRESENCE OF MALTOSE

Sugar present—glucose	Carbon dioxide production 75–15 min.	Glucose calculated[1]
mg.	*mm.Hg.*	*mg.*
600	317	600
540	294	556
480	252	478
420	220	417
360	180	341
240	113	214
120	59	112
60	31	59

Sugar present		Carbon dioxide production 75–15 min.	Glucose calculated[1]
Glucose	Maltose		
mg.	*mg.*	*mm.Hg.*	*mg.*
600	—	301	600
540	60	280	558
480	120	255	509
420	180	214	427
360	240	178	335
240	360	119	237
120	480	62	124
60	540	31	62

[1] Based on the assumption that glucose only is fermented and that a proportional relationship exists between mm.Hg. pressure and glucose present.

given in Table III. In every set of determinations the corresponding fermentation value for 600 mg. glucose was determined. The "calculated" quantity of glucose for any one manometer reading was derived by assuming an exactly proportional relationship between carbon dioxide produced and glucose present.

The data in Table III indicate that carbon dioxide production in the 1-hour interval following the first 30 minutes of fermentation is most closely related to glucose quantity over a range of sugar concentrations. The definite limitations in replicability of the procedure

likewise are evident. At best the method can be considered as only roughly quantitative. Also shown in Table III is further evidence that the presence or absence of maltose has no influence on the glucose calculation.

Determination of Maltase Activity. With a procedure available for estimating glucose in the presence of maltose it became possible to evaluate maltase activity. To bear some relationship to certain industrial applications it was desired to determine the extent of such activity occurring during a 1-hour period at temperatures in the neighborhood of 50°C. The procedure developed is as follows:

Three aluminum pressuremeter cups are used for each determination. Each contains 0.06 g. sodium chloride, 0.04 g. crystalline

TABLE III

RELATIONSHIP OF CARBON DIOXIDE PRODUCTION TO GLUCOSE QUANTITY DURING THE ONE-HOUR INTERVAL FOLLOWING THE FIRST 15, 30, 45, AND 60 MINUTES OF FERMENTATION

(Quantity of "Maca" yeast—1.5 g.)

Sugar present		Calculated glucose from pressuremeter readings for 1-hour intervals			
Glucose	Maltose	15 and 75 min.	30 and 90 min.	45 and 105 min.	60 and 120 min.
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
120	—	168	132	108	—
240	—	306	276	240	228
240	—	234	240	246	246
240	360	276	240	228	240
360	—	408	360	336	342
480	—	504	486	450	450
480	—	504	480	486	510
480	120	522	482	456	462

magnesium sulfate, 0.04 g. potassium dihydrogen phosphate, 0.011 g. potassium monohydrogen phosphate, 0.0008 g. thiamine hydrochloride, and 0.0008 g. pyridoxine in 15 ml. of solution (pH 5.8). To the first cup is added 600 mg. glucose and to each of the second and third cups 600 mg. maltose. Cups one and two are placed in a 50°C. water bath and brought to temperature (3 minutes), and the "blank" cup, No. 3, is placed in a boiling water bath. At appropriate intervals, aliquots of the enzyme solution to be evaluated are added to the three cups either in 10 ml. volume or accompanied by sufficient water to give a total cup volume of 25 ml. The enzyme aliquots should be such that 40% conversion of the maltose is not exceeded during the reaction, for reasons to be discussed later. Each cup is rotated rapidly upon addi-

tion of the enzyme to mix and to bring the contents rapidly to the desired temperature. Each cup is covered with a watch glass to minimize evaporation. After 3 minutes in the boiling bath the "blank" cup containing nutrient, maltose, and inactivated enzyme may be cooled and carried along with the enzyme tests.

After 1 hour at 50°C. each cup is placed in a boiling water bath for 3 minutes and is then cooled in running cold water. On a new selected time schedule the cups are brought to 30°C., 10 ml. of yeast suspension containing 1.5 g. of "Maca" yeast added to each, and the manometers affixed. The pressure is read in mm. mercury after 30 minutes of fermentation and again after 90 minutes. The carbon dioxide produced in the indicated time interval with the blank is subtracted from the interval readings found for the two cups containing active enzyme. The glucose produced in the cup is calculated as follows:

$$\frac{\text{Corrected maltose reading}}{\text{Corrected glucose reading}} \times 600 \text{ mg.} = \text{mg. glucose produced}$$

No detailed studies of the influence of time, temperature, or pH on the activity of any one fungal enzyme were made. The object of the investigation primarily was to evaluate the maltase activity of a wide variety of products under more or less rigidly stipulated conditions. The optimum activity conditions for one product might bear little relationship to those for another. The conditions used, 1 hour at 50°C. and pH 5.8, are known to be favorable if not optimum for the maltase activity of at least several fungal products.

Calculation of Maltase Activity. The data obtained by the procedure represent the glucose produced from 600 mg. maltose by an extract aliquot equivalent to a given weight of mold bran. For comparison of various materials it is requisite that this be proportional to maltase quantity.

In Fig. 1 is shown the relationship between the quantity of mold bran and the amount of maltose converted in 1 hour at 50°C. Two different molds are represented, a strain of *Aspergillus oryzae* and one of *A. niger*. The bran produced by culturing *A. oryzae* had about twice the activity of the *A. niger* bran.

It is apparent that maltose conversion is directly proportional to mold bran quantity only at low conversion values. Activity evaluations at one mold bran concentration may be compared by calculating the ideal conversions that would result from the first small increments of bran. If the two curves have the same characteristics over an appreciable range, one curve may be drawn relating the actual maltose

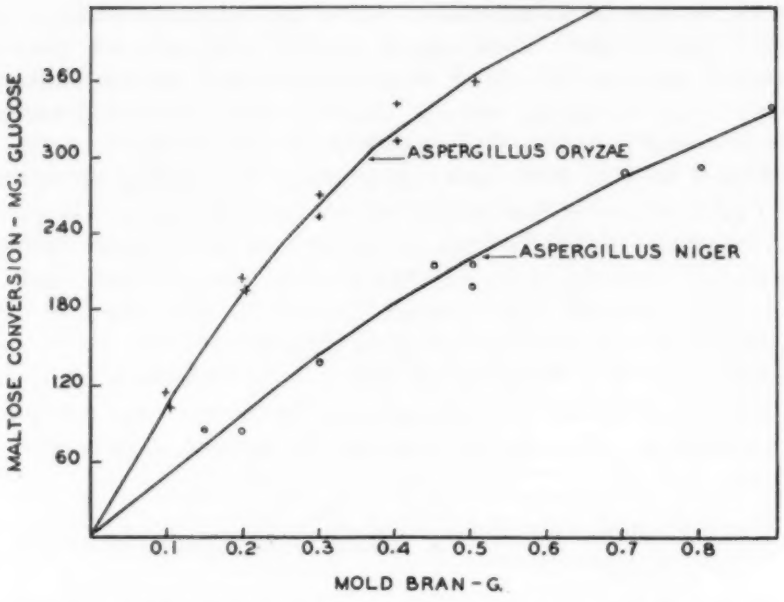

Fig. 1. Relation between maltose conversion and quantity of two mold brans.

converted to the conversion that would have resulted if the initial enzyme-maltose conversion relationship had persisted in an ideal linear fashion. For each of the mold brans the "found" values at any one concentration (taken from the curves of Fig. 1) were compared with the values that would have resulted if the initial bran-quantity

TABLE IV

RELATIONS BETWEEN "FOUND" AND "IDEAL" MALTOSE CONVERSION BY INCREASING QUANTITIES OF TWO MOLD BRANS

(Ideal conversions calculated by linear extrapolation of initial low-quantity conversions)

Wt. mold bran *A. oryzae*	Glucose found	Glucose ideal	Wt. mold bran *A. niger*	Glucose found	Glucose ideal
g.	*mg.*	*mg.*	*g.*	*mg.*	*mg.*
0.05	54	54	0.05	24	24
0.1	105	108	0.1	48	48
0.15	150	162	0.2	90	96
0.2	195	216	0.3	132	144
0.25	234	270	0.35	152	168
0.3	264	324	0.4	174	192
0.35	297	378	0.5	210	240
0.4	324	432	0.6	246	288
0.5	369	540	0.7	279	336
			0.8	312	384
			0.9	342	432
			1.0	369	480

maltose-conversion relationships had persisted in linear proportionality throughout higher concentrations. The results are given in Table IV and are shown diagrammatically in Fig. 2. The two curves essentially coincide up to about 40% conversion of maltose (240 mg. glucose produced). Accordingly, below this limiting value a "found" conversion value for mold bran may be "corrected" to a figure linearly proportional to enzyme quantity by referring to the curve. By using the calculated figure, a unit value for a mold bran may be determined that can be related to a similarly calculated value for another product. The arbitrary *maltase unit value* selected for the work is the *milligrams*

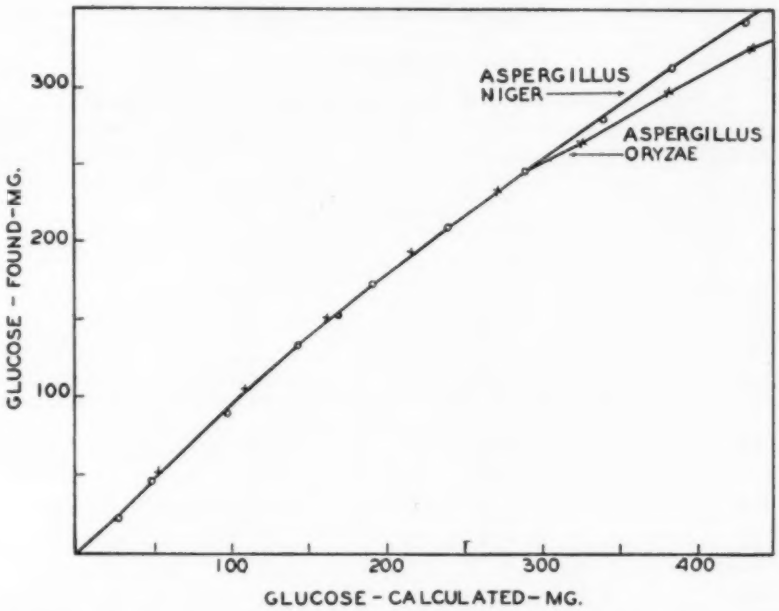

Fig. 2. Relation between "found" and "ideal" maltose conversions for increasing quantities of two mold brans. The "ideal" conversions are calculated by linear extrapolation of the relations found for initial small increments as shown in Fig. 1.

of maltose converted to glucose by the action of 1 g. of mold bran in 1 hour at 50°C. Observance of the reaction conditions is assumed, i.e., 600 mg. maltose as a substrate, pH 5.8, 25 ml. liquid volume, and a conversion limit of 40%.

The glucose present in the cup after the reaction is calculated from the fermentation data. By the use of the relationship shown in Fig. 2 the calculated amount of glucose representing linear proportionality between enzyme concentration and glucose production is determined. Based on the weight of dry enzymic material used for maltose conversion, the unit value can be obtained. For example, if an extract

aliquot equivalent to 0.5 g. mold bran (10% moisture) is used for the reaction and 200 mg. glucose is produced (corrected to 225 mg. by Fig. 2) the maltase activity of the material would be $\frac{225}{0.9 \times 0.5}$ or 500 units.

Maltase Activity of Mold Brans. The maltase evaluation procedure and method of calculation was applied to 41 experimental brans prepared by culturing various fungal isolates on wheat bran medium. Those brans along with their amylase activities were kindly supplied by Dr. George L. Peltier of the University of Nebraska. A description of the mold brans together with their amylase and maltase activities is given in Table V. Also included for comparison are the results for

TABLE V

AMYLASE AND MALTASE ACTIVITY OF VARIOUS MOLD BRANS

Fungal isolate[1]	Organism	Amylase activity[2]	Maltase activity
	Aspergillus type		
		min.	*units*
Commercial "A"	*oryzae*	1.0	850
FA 35	*oryzae*	2.3	297
FA 38	*oryzae*	2.9	265
FA 38	*oryzae*	3.4	255
Commercial "B"	*oryzae*	13.0	164
FA 36	*oryzae*	3.6	145
MF 309	black	180	415
MF 349–430	black	180	407
FA 10C	green	3.0	297
MF 19	green	19.0	255
MF 375	green	28	92
MF 347	yellow-gold	54	73
MF 417	yellow-gold	70	48
MF 30	tan	120	42
MF 321	tan	230	42
MF 155	clavate heads	1430	73
MF 85	clavate heads	1060	60
MF 377	miscellaneous	920	66
MF 413	miscellaneous	1020	60
MF 39	miscellaneous	72	48
MF 367	miscellaneous	70	36
MF 473	*Rhizopus-Mucor*	60	297
MF 469	*Rhizopus-Mucor*	75	205
MF 467	*Rhizopus-Mucor*	68	177
MF 426	*Rhizopus-Mucor*	83	140
MF 43	*Rhizopus-Mucor*	200	98
MF 672	*Rhizopus-Mucor*	218	66

TABLE V—*Continued*

Fungal isolate[1]	Organism	Amylase activity[2]	Maltase activity
	Penicillium type		
		min.	*units*
FA 1C	*Penicillium*	80	280
WF 265	blue-green	76	140
MF 49	blue-green	160	30
MF 275	gray-green	2000	135
MF 460	gray-green	880	98
MF 541	gray-green	990	66
MF 257	gray-green	730	30
F 14C	yellow-green	730	60
MF 509	*Alternaria*-like	160	98
MF 47	*Alternaria*-like	135	36
MF 202	mixed culture	660	36
MF 157	mixed culture	96	36
MF 255	unclassified	290	36
MF 7	unclassified	43	24
MF 76	unclassified	100	24
MF 495	Trichoderma	1640	12

[1] With the exception of the two commercial mold brans the designations are those supplied by Dr. George L. Peltier of the University of Nebraska and to be presented by him in a publication now in press.

[2] Amylase activity—dextrinizing time for 0.05 g. mold bran; amylase activity is inversely proportional to the values recorded.

two commercial mold brans. The amylase activities are not calculated as "unit" values but are given merely as "dextrinization time for 0.05 g. bran" (i.e., the time required for 0.05 g. mold bran to dextrinize 20 ml. of a 1% starch suspension at pH 5.0 and 30°C.). Accordingly, amylase activity is inversely proportional to the value given.

It is apparent that there is very little correlation between amylase activity and maltase activity, with the exception that the *Aspergillus oryzae* brans tended to be high in both activities. However, two mold brans cultured with *A. niger* isolates were exceeded in maltase activity only by one of the "commercial" products, notwithstanding that their amylase activities were exceeded by 26 of the 43 brans evaluated. There is an interesting relation between the activities of the two enzymes within a group classification. For example, in the *A. oryzae* and the *Rhizopus-Mucor* groups the order of activity is about the same for each enzyme, indicating a positive correlation between the amylase and maltase production capacities of a given organism.

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Application of the Procedure. One application of a method for maltase activity has been illustrated above—the testing of numerous mold brans prepared by culturing isolates with unknown potentialities. Most of the other investigations were not carried out under exactly the same conditions described here so they will not be outlined in detail. However, of the various materials tested, barley malt proved to have no measurable maltase activity. Of three "bacterial brans" tested, one commercial and two supplied by Dr. George L. Peltier, the commercial had no maltase activity and the two Nebraska isolates (W-96 and P-65) had about half the activity of those commercial mold brans available on the market. The several commercial mold brans tested, from various sources, had similar intermediate maltase activities comparable in magnitude to the "Commercial B" sample of Table V. The "Commercial A" mold bran of Table V was a special, very active product not available in large quantity.

The maltase procedure likewise proved of value in studies on the concentration of the mold bran carbohydrase system. A mold bran extract and dispersions of isopropyl and ethyl alcohol precipitates obtained from it were compared at equal amylase activities. The isopropyl alcohol precipitate had 76% as much maltase activity as the extract of original bran, whereas the ethyl alcohol precipitate had only 30% of the original maltase activity. This demonstrated that, at levels of alcohol satisfactory for amylase precipitation, isopropyl alcohol was more efficient than ethyl alcohol in the precipitation of maltase and its recovery in an active form.

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INFLUENCE OF SALTS ON AMYLASE ACTIVITIES OF MALT [1]

VICTOR L. ERLICH[2] and GEORGE M. BURKERT[3]

ABSTRACT

Salts added in small amounts to distilled water in the extraction of barley and wheat malts increase the alpha-amylase activities in the extract strongly but variably. At salt concentrations of approximately 0.1 *N* or less, respective optimum activities are reached, whereas further salt additions may depress the activity more or less, depending upon the specific ions present. Judicious selection of salts yielded highest optimum or "maximum" alpha values which are not increased by raising the temperature of extraction from 20° and 30° to 40°C., or by increasing the duration of extraction. These "maximum" alpha activities appeared to be characteristic of the respective malt, and close to those obtainable under industrial mashing conditions. The ratio of these "maximum" alpha activities to those determined in distilled water extracts varies considerably from one malt to the other.

Extraction of barley malt with dilute salt solutions had little or no effect on beta-amylase activity, whereas two wheat malts contained large quantities of salt-soluble beta-amylase. Higher salt concentrations may depress beta-amylase activity depending upon the specific ions, but to different extents than alpha-amylase.

The influence of the salts added before extraction was compared with that after extraction. At low salt concentrations no activation was found for alpha-amylase after extraction, while slight stimulations of beta-amylase occurred with some ions. The addition of high salt concentrations to the extracts decreased the activity of both alpha- and beta-amylase. The lower amylase values obtained when malts are extracted with salts of high concentrations are due to the inhibitory effects of the ions on enzyme activity rather than to lower solubility of these enzymes in such salt solutions.

The effect of papain upon the release of alpha-amylase appeared to be significantly different from that of latent beta-amylase.

The influence of proteolytic enzymes and salts on the extraction or release of the amylases from wheat and barley and from their malts has

[1] Manuscript received June 24, 1948.
[2] New York.
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long been known. It has been observed also that the activities of aqueous solutions of alpha- and beta-amylase are affected by the presence of electrolytes, especially of metal salts, the effect depending upon the nature of the ions, the concentrations, and the pH of the solution (1, 2, 12, 28, 29, 34–39). The alpha-amylases of both cereals and noncereals are partially protected against inactivation by heat, high acidity, and inhibitors by the presence of small amounts of calcium salts (8, 9, 16, 18, 20, 21, 23, 30, 40, 42). It has been reported that the liquefaction of grain mashes with malt is improved when calcium sulfate is added (41), and additions of calcium sulfate or calcium chloride and of sodium chloride have been recommended in the analytical determination of malt alpha-amylases to obtain higher and more reproducible results (13, 17, 19, 21, 43).

However, the precise effect of salts on the extraction of the amylases from malt has not been clearly established. Since inorganic salts in varying low concentrations are always present in the mash waters in the commercial conversion of starch, a study was undertaken to determine the effect of different salts, particularly of those found in industrial waters, upon the extraction of amylases from malt and also upon the activity of extracts made with distilled water.

Materials and Methods

The majority of the experiments were conducted with commercial brewers' and distillers' malts; the former are dried at final temperatures of 70° to 75°C. and the latter at about 50°C. In addition, green barley malts, dried in vacuo in the laboratory, and two wheat malts were examined. Each sample was thoroughly mixed and stored in airtight containers.

In principle, the methods used for extracting the malt, conducting the ensuing starch hydrolysis, and determining diastatic power followed those outlined in *Cereal Laboratory Methods* (4th ed. 1941), while dextrinizing activity was determined by the method of Sandstedt, Kneen, and Blish (32) as modified by Olson, Evans, and Dickson (25, 26) and by Redfern (31).

Ten grams of the finely ground malt were extracted with 200 ml. of distilled water or the particular salt solution under study at temperatures of 20°, 30°, and 40°C. for times varying from 10 minutes to 22 hours. Blank tests were made at least daily. The alpha-amylase activity was calculated in terms of Sandstedt, Kneen, and Blish units at 20°C. ("20° dextrinizing units") (26). The beta-amylase activity was calculated as the difference between the maltose equivalents of the

total saccharifying activity or diastatic power and that of the alpha dextrinizing activity. The equivalents given by Olson, Evans, and Dickson (25) were employed: They are in good agreement with those of other workers (13), between about 15 and 35 "20° dextrinizing units," but differ somewhat at lower and higher activities. The reliability of these beta-amylase values is naturally influenced by errors in both measurements from which they are calculated.

Results

Variations in Amylase Activities of Distilled Water Extracts of Brewers' Malt upon Storage. Subsamples from a uniform lot of one brewers' malt ("A") of medium kernel size were extracted with distilled water at 20° and 30°C. respectively after various intervals of storage up to 115 days and the alpha-amylase, diastatic power, and beta-amylase of the extracts determined at 20°C. The results given in Table I show that the alpha-amylase values vary considerably more than those for diastatic power, and consequently also more than beta-

TABLE I

AMYLASE ACTIVITIES DETERMINED ON ONE BREWERS' MALT AFTER VARIOUS INTERVALS OF STORAGE[1]

Age of sample	Extraction at 20°C.			Extraction at 30°C.		
	Alpha S.K.B. units	D.P.	Beta maltose equiv.	Alpha S.K.B. units	D.P.	Beta maltose equiv.
Days		°*L.*			°*L.*	
2	17.8	95	320	—	—	—
6	17.8	96	324	—	—	—
43	14.7	95	332	—	—	—
44	14.7	98	344	19.4	101	340
51	14.7	99	348	—	—	—
57	15.6	95	328	—	—	—
59	16.7	97	332	—	—	—
64	16.7	94	320	—	—	—
66	15.6	94	324	19.4	95	316
73	15.6	94	324	19.4	95	316
77	14.7	95	332	—	—	—
79	16.7	96	328	—	—	—
81	16.7	96	328	20.0	98	324
82	15.6	94	324	—	—	—
84	16.7	96	328	—	—	—
97	14.7	97	340	—	—	—
107	17.8	95	320	22.7	97	312
115	16.6	96	328	—	—	—
Mean	16.1	96	328	20.6	97	320
Standard deviation	1.12	1.41	8.0	1.43	2.5	11.4
Coefficient of variability, %	7.0	1.5	2.4	6.9	2.6	3.6

[1] Extraction with distilled water for 2 hours; hydrolysis at 20°C.

amylase. The variations in alpha-amylase are apparently not correlated with the duration of storage. The pH of the extracts varied from 5.5 to 5.8, but there was no apparent relation between these values and those for alpha-amylase activity.

The extracts made at 30°C. gave higher alpha-amylase activities than those prepared at 20°C., but increasing the extraction temperature had no marked influence on the beta-amylase values.

Influence of Various Salts on the Extraction of Amylases from Barley Malt. Several commercial barley malts, brewers' and distillers', were extracted with distilled water and with 0.001 to 2.0 *N* solutions of different salts and other agents, such as citric acid and calcium hydroxide, for 2 hours at 20° and at 30°C. The amylase activities were

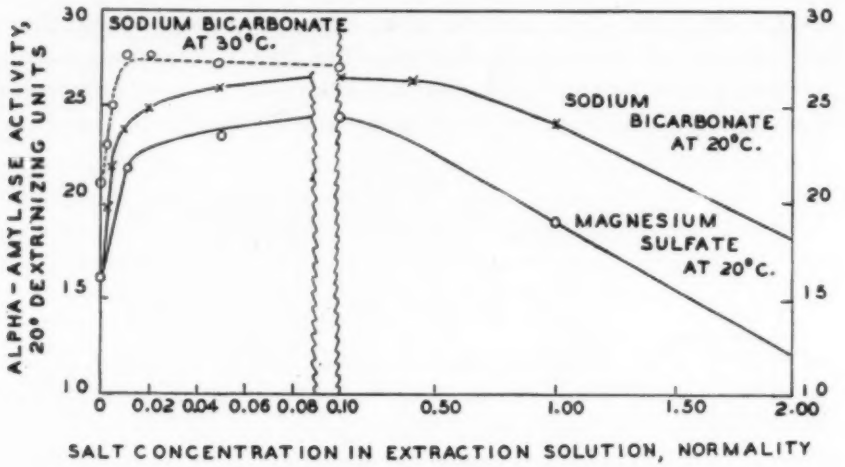

Fig. 1. Effect of salt additions on the extraction of alpha-amylase of one brewers' malt, at 20° and 30°C. for two hours.

determined at 20°C. for both temperatures of extraction. The concentrations were compared on normality basis whereby—in deviation from usual practice—the hydrogen ion of acid salts was considered equivalent to a metal ion; consequently, bicarbonates and primary or secondary phosphates were calculated as di- and tri-valent respectively.

The smallest salt additions influenced the diastatic power, but much more the alpha-amylase activities.

The effect on alpha-amylase activity showed the same pattern for all the malts tested and all the salts used. These activities are sharply increased in the lowest bracket of salt concentrations. They reach optimum values below or at about 0.1 *N*, and decline more or less at higher concentrations. This is illustrated in Fig. 1 by typical curves

which show the effects of increasing salt concentrations of sodium bicarbonate and magnesium sulfate on alpha-amylase activity.

Other salts tested on the same malt ("A") yielded optimum levels of alpha-amylase which varied between those of the two examples, but none gave a higher optimum activity than sodium bicarbonate.

To provide a common basis for comparing the results with different malts, the alpha-amylase activities were expressed in per cent of the "maximum" value, that is the value obtained for each malt by extraction with 0.1 *N* sodium bicarbonate at 30°C. for 2 hours. The diastatic power and beta-amylase values were expressed in per cent of the corresponding activities for the extracts made with distilled water at 20°C. The results, when calculated on this basis, showed a marked similarity for the different barley malts extracted with the same salt solutions. For the brewers' malts investigated in this study, these per cent values were found to be practically identical, except at concentrations below about 0.01 *N* (especially in the most dilute solutions).

Representative results obtained with three brewers' malts and several selected concentrations of the various substances are given in Table II. These brewers' malts had the following characteristics:

Origin	*Diastatic power* °*L.*	*"Maximum" alpha-amylase activity units*
"A," South Dakota	96	28
"B," North Dakota	127	33
"C," Minnesota	137	40

The alpha-amylase activities were increased to within about 5% of the "maximum" value by appropriate concentrations of the first eight salts; the other substances gave increases which were 10% or more below the "maximum." In all cases the lowest salt concentrations gave higher values when the extractions were conducted at 30° than at 20°C. As the concentrations were increased, the differential effect of extraction temperature became less pronounced and practically disappeared at about 0.1 *N*, the concentration at which the respective highest activities were reached.

Relatively insignificant increases in beta-amylase activity occurred with any of the salts, in contrast to their very marked effects on alpha-amylase. Some salts such as the alkali chlorides and sulfates and magnesium sulfate were without appreciable effect on beta-amylase activity in concentrations up to 2.0 *N*, whereas others such as the nitrates, acetates, and bicarbonates of sodium and potassium, and more particularly calcium and magnesium chloride and sodium sulfo-

TABLE II

EFFECT OF EXTRACTING BREWERS' MALT WITH VARIOUS SOLUTIONS ON AMYLASE ACTIVITIES[1]

Extraction medium	Salt concentration	Alpha-amylase extracted at		Diastatic power extracted at		Beta-amylase extracted at		pH of extract
		20°	30°	20°	30°	20°	30°	
	N	%	%	%	%	%	%	
Distilled water	—	58	74	*100*	102	*100*	98	5.5–5.8
Sodium bicarbonate	0.002	69	81	105	110	104	105	6.2
	0.005	81	89	110	110	106	103	6.6
	0.01	85	*100*	110	113	103	103	7.0
	0.05	89	96	106	116	99	107	7.7
	0.10	94	94	109	117	100	107	7.9
	1.00	85	89	98	97	88	87	8.2
	2.00	64	56	95	98	91	94	8.3
Potassium bicarbonate	0.01	84	94	—	113	—	103	6.2
	0.05	87	94	107	117	98	108	6.9
	0.10	90	94	—	117	—	108	7.3
	0.50	87	90	104	112	96	104	8.0
	2.00	—	83	—	97	—	88	8.2
Sodium nitrate	0.01	76	80	106	107	100	101	5.8
	0.05	91	92	106	107	99	100	5.8
	0.10	100	92	106	107	96	99	5.8
	1.00	91	96	104	105	95	92	5.8
	2.00	89	89	105	100	95	90	5.8
Sodium acetate	0.01	75	83	114	—	106	—	5.7
	0.10	92	92	106	108	104	107	6.1
	0.50	100	89	—	—	—	—	6.7
	2.00	53	58	71	75	72	75	7.2
Disodium hydrogen phosphate	0.01	89	89	110	110	105	105	6.8
	0.10	92	92	112	112	107	107	7.3
	0.50	89	89	112	112	108	108	7.8
Diammonium hydrogen phosphate	0.01	89	92	103	108	97	103	6.3
	0.10	92	100	107	110	101	103	7.3
	0.50	92	92	105	106	99	102	7.4
	2.00	75	75	76	82	68	76	7.9
Sodium sulfocyanate	0.01	81	85	105	108	96	97	5.8
	0.05	95	95	104	106	93	96	5.75
	0.10	95	95	105	108	96	97	5.7
	1.00	81	49	59	38	45	28	5.7
Sodium citrate	0.01	83	—	107	—	102	—	6.2
	0.05	94	—	107	—	98	—	6.6
	0.10	91	—	106	—	98	—	6.7
	1.00	65	—	93	—	89	—	7.1

[1] Extraction for 2 hours at 20° and at 30°C. Amylase determinations in extracts at 20°C. Alpha-amylase calculated in per cent of respective "maximum" value, diastatic power and beta-amylase in per cent of "distilled water" values.

TABLE II—*Continued*

Extraction medium	Salt concentration	Alpha-amylase extracted at		Diastatic power extracted at		Beta-amylase extracted at		pH of extract
		20°	30°	20°	30°	20°	30°	
	N	%	%	%	%	%	%	
Chlorides and sulfates of sodium, potassium, and ammonium	0.002	63	63	101	101	100	100	5.7
	0.005	73	73	104	104	100	100	5.7
	0.01	78	78	105	107	100	102	5.7
	0.05	84	84	109	109	101	101	5.7
	0.10	84	84	110	108	102	100	5.7
	1.00	90	85	107	106	100	100	5.6
Potassium chloride	2.00	85	85	108	114	100	106	5.4
Sodium chloride	2.00	71	69	—	103	—	99	5.3
Potassium sulfate	2.00	64	59	95	99	93	99	5.3
Ammonium sulfate	2.00	47	53	94	90	90	96	5.4
Calcium acetate	0.01	71	76	103	105	100	102	6.8
	0.10	84	84	108	109	101	103	6.5
	1.00	77	80	97	98	95	91	6.7
	2.00	71	74	97	96	91	89	6.9
Calcium chloride	0.01	72	79	103	106	100	102	5.4
	0.10	79	83	103	107	99	103	5.6
	0.50	77	81	—	—	—	—	6.1
	1.00	77	80	102	101	96	93	6.3
	2.00	53	53	24	11	14	0	7.0
Magnesium chloride	0.01	75	92	—	—	—	—	5.5
	0.10	79	89	111	112	108	108	5.4
	0.50	83	89	—	—	—	—	4.8
	2.00	50	21	72	46	71	49	4.6
Magnesium sulfate	0.01	78	85	102	110	97	100	5.5
	0.05	84	87	105	108	96	102	5.4
	0.10	89	91	100	108	94	101	5.3
	1.00	67	62	98	96	95	94	5.3
	2.00	44	47	88	87	90	88	5.6
Potassium dihydrogen phosphate	0.01	68	75	99	102	98	103	5.6
	0.10	75	78	105	101	103	100	5.3
	0.50	75	75	103	101	100	99	4.9
	2.00	33	0	97	86	105	101	4.4
Citric acid	0.005	31	—	85	—	94	—	4.6
	0.01	0	—	52	—	61	—	4.3
	0.05	0	—	4	—	4	—	3.1
Calcium hydroxide	0.02	89	89	86	60	73	42	8.5
	0.04	89	89	19	19	0	0	9.4
	0.10	74	74	17	15	0	0	11.2
Calcium peroxide	0.01	81	—	91	—	81	—	6.4
	0.05	81	—	17	—	0	—	8.6
Ammonium persulfate	0.01	56	—	91	—	88	—	5.2
	0.05	18	—	67	—	73	—	4.8
Potassium periodate	0.01	25	—	6	—	0	—	4.9
	0.05	0	—	0	—	0	—	4.15

cyanate, reduced the activity at the higher concentrations, especially when the extractions were made at 30°C.

The increase in diastatic power, which represents the combined saccharifying activities of both amylases, at low salt concentrations is due, primarily or exclusively, to the increase in alpha-amylase. At the higher salt concentrations, the decrease in diastatic power is influenced by the depressing action of the salts on either amylase or on both.

Effect of Various Salts on the Activity of Malt Amylases in Solution. Samples of a brewers' malt ("A") were extracted with distilled water at 20° and 30°C. for 2 hours and the solutions filtered. Various salts were then added to give the same series of concentrations as were used in the extraction experiments outlined in the previous section. The amylase activities were determined at 20°C. and the results expressed in per cent of the "distilled water" values without any salt addition. These per cent values were compared with the corresponding extraction figures in which the alpha-amylase activity was again expressed in per cent of the "maximum" activity and the beta-amylase in per cent of the "distilled water" extraction value at 20°C. The results are recorded in Table III.

For alpha-amylase no marked activation occurred when the salts were added after extraction; thus the strong increases of the extraction values could not be due to an activating influence of the salts upon the enzyme. Some salts depressed the activity of the solution even at concentrations below 0.1 *N*, and many salts had a pronounced depressing effect at higher concentrations of 1.0 to 2.0 *N*. These inhibitory effects were in good agreement with those observed in extraction, as can be seen from the "maximum" alpha-amylase values. For some salts, sodium chloride for instance, the "maximum" extraction activities were not obtained, although they showed no depressing effect on the solution. However, sodium chloride gave "maximum" extractions when employed on some other types of malts, wheat malt for instance, and also when the time of extraction was increased, as will be shown later.

For beta-amylase the position is a different one, especially at concentrations below about 0.5 *N*. Some salts, such as bicarbonates and acetates, somewhat stimulated the activity of the solution, and this influence appeared to be reflected in corresponding increases of the extraction figures. Higher concentrations were predominantly depressing, but in most instances differently than for alpha-amylase.

Effect of Natural Waters and of Papain on the Extraction of Barley Malt Amylases. It was of interest to compare the effects of salts

TABLE III

Comparative Effects of Salts Added Before and After Extraction on Amylase Activities of Extracts[1]

Salt	Concentration	Alpha-amylase activity				Beta-amylase activity			
		20°C.		30°C.		20°C.		30°C.	
		Before extr.	After extr.	Before extr.	After extr.	Before extr.	After extr.	Before extr.	After extr.
	N	%	%	%	%	%	%	%	%
Sodium chloride	0.05	85	100	85	—	100	100	100	—
	0.5	85	100	85	—	100	100	100	—
	1.0	90	100	85	—	100	100	100	—
	2.0	70	—	70	(85)[2]	—	—	99	(90)
Sodium nitrate	0.05	90	100	90	100	100	100	100	100
	0.5	95	100	90	—	100	95	100	—
	1.0	90	100	95	90	95	90	90	85
	2.0	90	—	90	(85)	95	—	90	(90)
Sodium acetate	0.05	90	—	90	105	105	—	105	105
	1.0	85	—	85	90	90	—	90	90
	2.0	55	—	60	(70)	70	—	75	(75)
Sodium bicarbonate	0.05	90	—	95	100	100	—	105	115
	1.0	85	—	90	75	90	—	90	95
	2.0	65	—	55	(70)	90	—	90	(80)
Sodium citrate	0.2	90	90	—	—	100	110	—	—
	1.0	65	—	—	—	90	—	—	—
Potassium dihydrogen phosphate	2.0	35	—	0	(0)	105	—	100	(105)
Diammonium hydrogen phosphate	2.0	75	—	75	(85)	70	—	75	(75)
Calcium acetate	0.01	70	95	75	—	100	100	100	—
	0.05	80	—	85	80	100	—	105	105
	0.2	85	80	85	—	100	110	100	—
	1.0	75	—	80	70	95	—	90	90
	2.0	70	—	75	(80)	90	—	90	(90)
Calcium chloride	0.05	75	—	85	95	100	—	100	100
	1.0	75	—	80	85	95	—	95	30
	2.0	55	—	55	(65)	14	—	0	(8)
Magnesium sulfate	0.05	85	95	85	90	95	105	100	100
	0.5	85	90	85	—	95	100	100	—
	1.0	65	85	60	65	95	100	95	100
Sodium sulfocyanate	0.05	95	—	95	105	95	100	95	100
	1.0	80	—	50	25	45	—	30	5
Potassium iodate	0.05	0	—	0	0	0	—	0	—

[1] Extractions for 2 hours at 20° and 30°C. Amylase determinations in extracts at 20°C. Alpha-amylase for salts added *before* extraction in per cent of respective "maximum" extraction values; all other activities in per cent of "distilled water" values.

[2] Values in parentheses were measured on amylase solutions obtained after extracting one wheat malt (see below).

added to the distilled water before extraction with the influence of natural or industrial waters upon the extraction of malt.

A brewers' malt was extracted with: (a) distilled water, (b) a city water containing 280 p.p.m. total minerals mostly in the form of calcium and magnesium bicarbonate (average normality of 0.007), (c) a well water containing 1,350 p.p.m. total minerals, about one-half of which were bicarbonates and the remainder mainly calcium sulfate (average normality 0.027), (d) sodium bicarbonate and calcium acetate solutions of the same normalities as the two samples of water, and (e) 1% papain solution in water and in 1.0 *N* calcium chloride

TABLE IV

EFFECT OF EXTRACTING BREWERS' MALT WITH NATURAL WATERS AND WITH PAPAIN IN DISTILLED WATER ON AMYLASE ACTIVITIES[1]

Extraction medium	Alpha-amylase		Diastatic power		Beta-amylase		pH of extract
	20°C.	30°C.	20°C.	30°C.	20°C.	30°C.	
	%	%	%	%	%	%	
Distilled water	58	74	*100*	102	*100*	98	5.5–5.8
Calcium acetate, 0.007 *N*	67	76	102	104	100	101	6.6
City water, 0.007 *N*	78	89	107	121	102	114	6.4–6.7
Sodium bicarbonate, 0.007 *N*	82	91	110	111	106	103	6.8
Sodium bicarbonate, 0.01 *N*	85	*100*	110	113	103	103	7.0
Sodium bicarbonate, 0.027 *N*	89	97	108	115	102	106	7.4
Well water, 0.027 *N*	79	89	113	117	108	109	6.7
Calcium acetate, 0.027 *N*	78	84	106	109	101	102	6.7
Papain, 1% emuls.	79	80	126	132	122	130	5.3–5.5
Papain in 1.0 *N* calcium chloride	79	79	124	119	120	115	5.3
Calcium chloride, 1.0 *N*	77	80	102	101	96	93	6.3

[1] Extraction of brewers' malt "A" for 2 hours at 20° and 30°C. Amylase determination in extracts at 20°C. Alpha-amylase is expressed in per cent of "maximum" activity, diastatic power and beta-amylase in per cent of "distilled water" values.

solution respectively. The results of pH and amylase activity determinations are recorded in Table IV.

The alpha-amylase activities of both natural water extracts fell between those of the two salts. For the city water they were closer to bicarbonate, whereas for the well water they were closer to those for the calcium salt; this agrees with the respective contents of these mineral salts. For beta-amylase, a slightly higher stimulation was found than would be expected on the basis of the bicarbonate content.

With a 1% papain extraction, the beta-amylase activity was, as expected, much more strongly increased than by any salt. For alpha-amylase the picture is an entirely different one: only about 80% of the "maximum" value was reached. When 1% papain was added to a

1.0 *N* calcium chloride solution, the alpha-amylase activity of the latter was not changed, but the beta-amylase was strongly increased close to but somewhat below that of the papain-distilled water emulsion, the calcium chloride exerting its slight depressing effect. This may indicate that commercial papain improves the release of alpha-amylase only by adding soluble salts to the extraction solution, but without any release from a bound or inactive form as is well known to be the case for beta-amylase.[4]

Effect of Temperature and Time of Extraction. Further experiments were conducted at an extraction temperature of 40°C. using the same

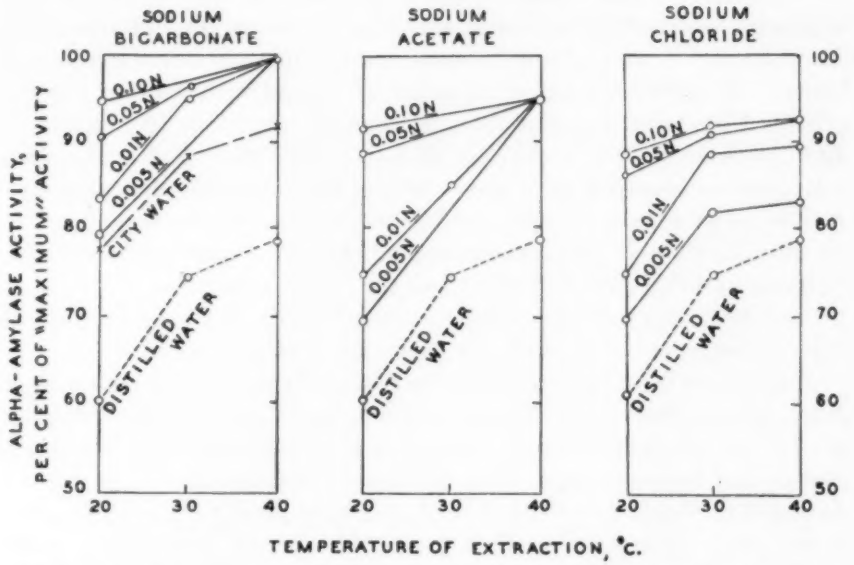

Fig. 2. Effect of temperature on the extraction of alpha-amylase of brewers' malts, at various salt concentrations. Extraction for two hours at 20° and 30°C., for one hour at 40°C.

series of salt concentrations employed in previous extractions at 20° and 30°C. The amylase activities were measured at 20°C.

The alpha-amylase activities were higher than at 30°C. in the lowest concentration bracket. Optimum activities were again obtained below or at about 0.1 *N*, but no activity was found to exceed the "maximum" alpha-amylase determined for the respective malts at 30°C. with suitable salts. Only with salts such as sodium chloride, which did not yield these "maximum" values at the lower temperature, were improvements noted. Different barley malts responded similarly to the increased temperature of extraction.

[4] Subsamples of the papain (Merck), extracted for 2 hours at 20° and 30°C. with distilled water, gave an average of 70.6% total soluble matter and 8.7% soluble mineral ash. This means that the filtered extract contained 0.087% inorganic minerals (ashed) coming from the papain, corresponding to an approximately 0.02 *N* salt solution (see Table II).

This is illustrated in Fig. 2. Sodium bicarbonate is an example of salts which gave the "maximum" alpha-amylase activities, at 0.1 *N*, at 20° and 30°C. within a margin of about 5%. Sodium acetate reached the "maximum" very closely at the lower temperatures, but completely at 40°C. even at the lowest salt concentrations. Sodium chloride remained below the "maximum" at 40°C., particularly at suboptimal salt concentrations. The respective values obtained with "city water" of 0.007 *N* were between those of sodium chloride and sodium bicarbonate. "Distilled water" acted as the very dilute salt solution it actually forms during the extraction of malt.

A similar picture resulted when the duration of extraction was increased. Sodium bicarbonate gave the "maximum" alpha-amylase value at 30°C. after about 1 hour, and no further increase up to 4 hours. It yielded the same "maximum" when the extraction was accelerated by agitation, in a Waring Blendor, after 5 to 10 minutes at 40°C. with no further increase in 15 and 20 minutes. Extraction with a 1% papain emulsion gave about 80% of the maximum alpha-amylase activity after 2 hours at 20°C., and 85 to 95% after 22 hours, depending on the malts tested, whereas extraction with 0.1 *N* sodium chloride for 22 hours at 40°C. gave 95 to 100% of the "maximum."

For beta-amylase, the temperature of 40°C. gave signs of slight destruction, especially when agitation was applied.

Variations of the pH of the extract from about 5 to 8 did not appear materially to alter either the alpha- or beta-amylase activities. Higher acidity or alkalinity caused the well-known differential effects with alpha- and beta-amylase. For purposes of illustration, results are recorded in Table II for citric acid, primary phosphate, and calcium hydroxide. Although calcium hydroxide is an extracting agent similar to other calcium compounds, it caused a small loss in alpha-amylase activity at pH 11, whereas the beta-amylase activity was already badly affected at pH 8.5. Conversely, citric acid destroyed alpha-amylase activity completely below pH 4.5, but only part of the beta-amylase activity.

Oxidizing agents have a destructive effect upon both amylases, but it is more pronounced for beta-amylase activity. Calcium peroxide, for instance, increased the alpha-amylase activity about the same degree as did calcium hydroxide, but destroyed beta-amylase at lower pH values. These and other data for ammonium persulfate and potassium periodate are recorded in Table II. Any possible influence of such chemicals upon the starch reagent (4, 5, 44) and upon the ferricyanide titration was taken into account by adequate blank tests.

The effect of reducing salts, such as sulfocyanate, had been studied in connection with starch hydrolysis (6, 12). Sodium sulfocyanate was

found to be a good extracting agent for alpha-amylase, but had no apparent activating influence upon beta-amylase (Tables II and III). In concentrations higher than 0.2 *N*, it caused destruction of both amylases.

Influence of Various Salts on the Extraction of Amylases from Wheat Malt. The effects on amylase activity of extracting wheat malt with various solutions at different temperatures are recorded in Table V. Although this study was not sufficiently extensive to permit definite conclusions for wheat malts in general, it was of interest to compare the results with those described above for barley malt.

For alpha-amylase the results were closely similar to those for barley malt; "maximum" activities were reached within the same range of salt concentrations.

TABLE V

EFFECT OF EXTRACTING WHEAT MALT WITH VARIOUS SOLUTIONS ON AMYLASE ACTIVITIES[1]

Extraction medium	Salt concentration	Alpha-amylase activity extracted at			Beta-amylase activity extracted at		
		20°C.	30°C.	40°C.	20°C.	30°C.	40°C.
	N	%	%	%	%	%	%
Distilled water	—	84	89	94	100	104	105[2]
		79[3]	94[3]	—	100[3]	122[3]	131[3]
City water	—	89	95	—	109	112	—
Papain, 1%	—	89	—	—	247	—	—
		88[3]		86[3]	237[3]	—	288[3]
Sodium bicarbonate	0.1	95	100	94	158	164	160
		100[3]	100[3]	100[3]	—	163[3]	—
	0.5	95	95	94	—	—	—
		94[3]	94[3]	—			
	2.0	95	80	—	100	87	—
Sodium chloride	0.1	100	100	94	155	167	167
		88[3]	100[3]	—	169[3]	174[3]	—
Sodium acetate	0.1	89	89	—	144	149	—
Calcium chloride	0.1	84	95	—	148	148	—
	0.5	73	80	—	93	92	—
	2.0	53	53	—	0	0	—

[1] Extraction for 2 hours at 20° and 30°C., for 1 hour at 40°C. Amylase determinations in extracts at 20°C. Alpha-amylase calculated in per cent of "maximum" activity, beta-amylase in per cent of "distilled water" values.

[2] Wheat malt "D" having a "maximum" alpha activity of 33 "20° dextrinizing units," a diastatic power of 106°L.

[3] Wheat malt "E" having a "maximum" alpha activity of 18 "20° dextrinizing units," a diastatic power of 57°L.

TABLE VI

RELATIONSHIP BETWEEN "MAXIMUM" AND "DISTILLED WATER" ALPHA-AMYLASE VALUES FOR DIFFERENT MALTS

Nature of malt[1]		Moisture of sample	Diastatic power	Alpha-amylase[2]		
				"Maximum" dextrinizing units	"Distilled water"	
					Dextrinizing units	Per cent of "maximum"
		%	°L.			
Barley brewers'	1	3.9	96	28	16	57
	2a	4.5	127	33	21	64
	2b	4.4	136	39	25	64
	3a	5.0	141	32	20	63
	3b	5.0	149	36	21	58
	4	4.6	125	31	17	55
	5	4.0	110	36	21	58
	6	4.0	121	29	18	62
	7	4.8	146	36	23	64
Barley distillers'	8a	6.3	184	47	23	49
	8b	6.4	179	46	23	50
	8c	5.5	161	40	27	68
	8d	5.8	163	43	28	65
	9a	6.5	196	44	24	55
	9b	6.2	193	43	21	49
	9c	6.1	182	43	20	47
	9d	6.3	194	43	22	51
	9e	7.1	190	47	24	51
	9f	6.7	192	49	29	59
	9g	6.5	184	47	26	55
	10	6.2	182	32	18	56
	11	6.8	188	38	22	58
	12	6.1	232	32	16	50
	13	7.9	186	30	21	70
	14	6.4	191	47	29	62
Barley green[3]	15	8.7	—	35	27	77
	16	7.2	—	47	35	74
	17	7.4	148	33	19	58
	18	7.6	219	52	27	52
Wheat malt	19	11.4	57	18	14	78
	20	7.9	106	33	27	82

[1] Samples designated with the same number but with different letters represent malts of the same shipment but processed under different conditions.
[2] "Maximum" alpha-amylase activity determined by extracting with 0.1 *N* sodium bicarbonate at 30°C. for 2 hours; "distilled water" activity by extracting at 20°C. for two hours, activities in extracts measured at 20°C.
[3] Dried in vacuo.

For beta-amylase, however, small additions of salts increased the diastatic power much more than for barley malts, thus indicating the presence of considerable amounts of salt-soluble beta-amylase.

Higher salt concentrations gave decreases for both amylases, as had been found with barley malt. The effect of salt additions to wheat

amylases in solution was strikingly similar to that shown for the barley malt amylases (see Table III).

Relationship between the "Maximum" Alpha-Amylase Activities and the "Distilled Water" Activities of Different Malts. From the foregoing studies, it appeared that one "maximum" alpha-amylase activity could be determined by extracting any malt with (0.05 to) 0.1 *N* sodium bicarbonate solution at 30°C. for (1 to) 2 hours. These "maximum" activities were determined on a wide variety of malts comprising barley brewers', distillers', and green malts, and two wheat malts. For each sample the "distilled water" activity was also determined after extraction at 20°C. for 2 hours. The results are shown in Table VI.

For the nine brewers' malts, the distilled water values for alpha-amylase activity only varied from 55 to 64% of the corresponding "maximum" values. For the other types of malts, however, a much wider spread (47 to 82%) was found.

Discussion

The release of *alpha-amylase* from barley and wheat malt is extremely sensitive to the presence of small amounts of salts. When malts are extracted with distilled water, it must be considered that the grain minerals are partly water-soluble. In the usual 5% malt infusion, one-tenth of the ash content of 2 to 3% is sufficient to add about 100 p.p.m. of mineral salts to the water, and the extraction would actually be made with a 0.001 to 0.002 *N* salt solution. Quantitative and qualitative variations of the grain ash, depending on the grain variety and on the method of malting and drying, can cause differences in the alpha-amylase activities measured in distilled water extracts. It has indeed been reported that more concentrated malt mashes give higher amylase activities (19, 43). It remains to be seen whether the presence of traces of electrolytes is essential for the activity of cereal alpha-amylase, as seems to be the case for alpha-amylases of other origin (9, 29, 34).

Alpha-amylase activities of barley malts show some correlation with their calcium and magnesium content (22), and this activity is also distinctly higher in mill-stream wheat flours which are richer in ash (7). In such cases, the grain minerals may have influenced, at least partly, the activity of the distilled water extracts used for the analyses.

When salts are added to the extraction water, the influence of small variations in the mineral content of the sample disappears. This is particularly true at the concentration of about 0.1 *N*, where optimum alpha-amylase activities were found for all salts examined, and "maxi-

mum" activities with certain salts. It has been reported that barley malts extracted with a 0.4 *N* sodium chloride solution gave in the filtrates alpha-amylase activity the same as or slightly lower than the infusion of these malts in distilled water without filtration (43). In this study such salt extractions yielded extracts which showed 85 to 100% of the "maximum" activities. The increases in alpha-amylase caused by salt additions have been reported to vary in a wide range, up to 82% of the "distilled water" extracts (19, 24, 27); in the present study increases up to 110% were found (Table VI).

The extraction conditions which gave the "maximum" alpha-amylase activities approximate those which prevail in breweries and distilleries. Bicarbonates and sulfates are usually the predominating anions, and calcium and magnesium the principal cations contained in industrial mash waters. Such waters (see "city water" in Fig. 2) gave, at 40°C. after 1-hour extraction, 90 to 95% of the "maximum" activity of the respective malt.

These results do not constitute definite proof that the "maximum" values are the true potential alpha-amylase activities, but they may be more representative for each malt than those obtained with other extractions, especially with distilled water.

The release of the "latent" *beta-amylase* from malts is significantly different from the effect of inorganic salts on alpha-amylase. This is clearly apparent when the action of papain on alpha- and beta-amylase is compared. Whereas its effect on beta-amylase exceeds by far that of any salt, the reverse is true with alpha-amylase. With the latter, no proteolytic actions or reactions involving the sulfhydryl group of the enzyme molecule were apparent. In barley and wheat grains part of the latent beta-amylase can be activated by salts (14, 15, 17, 33). Such salt-soluble beta-amylase was not found in any substantial amounts in the barley malts here examined, but very large amounts were determined in two wheat malts. This is reflected in the effect of small salt additions upon the total saccharifying activity. It is known that salts cause relatively small increases of diastatic power in barley malts (3, 10, 13, 27); the influence is, however, considerable for wheat malts.

Concurrent with the release of the amylases from malt during extraction, salts also affect the activities of both *enzymes in their water solutions*. In low concentrations, only beta-amylase was found to be stimulated by some salts. At higher salt concentrations, a pronounced depressing action upon the activity of the enzyme in solution overshadows more and more the effect of salts on the extraction of the amylase enzymes. These depressing effects are specifically different for the individual ions, and are not identical for alpha- and beta-

amylase. No marked variation was found, however, between barley and wheat malt amylases. This should be noted in connection with reports describing the conditions for the differential stability of the two amylases in wheat, on the one hand, and in barley malt, on the other (18, 21).

The general effects of the different ions in 2.0 *N* solution (pH 5 to 8) upon the activities of the two amylases in solution are summarized in Table VII.

TABLE VII

EFFECT OF INDIVIDUAL IONS ON ALPHA- AND BETA-AMYLASE IN WATER SOLUTION, WITHIN A pH RANGE OF 5 TO 8

Ions in solution	Alpha-amylase		Beta-amylase	
	Ion concentration		Ion concentration	
	Approx. 0.1 *N*	1.0–2.0 *N*	Approx. 0.1 *N*	1.0–2.0 *N*
Sodium	0[1]	–	0	0
Potassium	0	0	0	0
Ammonium	0	–	0	–
Calcium, magnesium	–	–	0	–
Chloride	0	–	0	—
Nitrate	0	0	0	–
Acetate	0	–	+	–
Sulfocyanate	0	—	0	—
Sulfate	0	—	0	0
Bicarbonate	0	—	+	0
Phosphate	0	–	+	+
Citrate	–	—	+	–

[1] "0" means: No significant influence.
"–" means: Activity slightly decreased.
"—" means: Activity more decreased.
"——" means: Activity strongly decreased.
"+" means: Activity slightly stimulated.

These approximate indications agree in many, but not in all, instances with work already published. Previously (12, 37, 38, 39), only the saccharogenic activities had been measured and compared for both amylases. Other differences may be explained by the fact that in the present study the amylase solutions were strongly diluted before the measurement of their activities, in accordance with usual practice. When the starch hydrolysis is conducted in the presence of salts in much higher concentrations (12), the results may be different.

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INFLUENCE OF SMALL QUANTITIES OF THIAMINE ON BAKING QUALITY OF WHEAT FLOUR [1]

H. M. R. HINTZER [2]

ABSTRACT

The influence of small quantities of thiamine on the baking quality of flour was investigated with the aid of extensograph, fermentograph, and baking experiments, using flours differing both in extraction and in the composition of the grist from which they were milled.

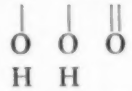
Thiamine added in concentrations up to 12 mg. per kg. of flour had no influence on the elastic properties of dough. In concentrations up to 10 mg. per kg. of flour it, apparently, exercised a slight stimulating action on the fermentation.

Even when variations were made in the type of flour, yeast concentration, dough consistency, and fermentation time, it was not possible to reproduce the improvement in the bread volume of 6.8% and 15.7% claimed in Dutch patent No. 55774 for the addition of 3 and 6 mg. respectively of thiamine per kg. of flour.

In the concentrations used, it is concluded that thiamine does not possess any improving action in breadmaking.

It is well known that very small concentrations of various oxidizing substances, such as potassium bromate, potassium iodate, and ammonium persulfate, exercise an improving influence on the baking quality of wheat flour. The extensive investigations and the many theories evolved to explain the behavior of these substances have been summarized by Sullivan *et al.* (8) and by Shen and Geddes (7). The thesis of Holger Jørgensen (3) is also a worth-while contribution to the elucidation of this problem.

Certain organic reducing agents such as ascorbic acid, reductic acid, and related substances, which, together with the oxidized form, constitute a redox-system can exercise a similar action to the above-mentioned oxidizers. From experiments in which ascorbic acid and a number of substances of analogous structure were compared as to their effect on the baking quality of wheat flour, Elion (1) was of the opinion that the group —C=C—C— performs an essential function in the improve-



ment of baking quality. Sandstedt and Hites (6) showed that the presence of the specific reducing group only fulfills one requirement.

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A second condition is that the oxidases in the flour must be able to form a redox-system from the reducing substance and its oxidized form. This system catalyzes the transfer of oxygen contained in the dough to the oxidizable groups in the protein components of the flour, where the oxidation by bromate and similar substances also takes place. Thus the action of both groups of substances can be reduced to the same principle. Maltha (4) has arrived at a similar point of view.

Regarded in this light, it is rather surprising to find in a patent by Hoffmann-La Roche (2) an improvement of baking quality attributed to thiamine, a chemically different type of substance, which does not possess the above-mentioned characteristic properties. According to the specification the addition of 3 and 6 mg. thiamine per kg. of flour exerts a volume improvement of 6.8 and 15.7% respectively.

Moran and Drummond (5), investigating the properties of white flour enriched with thiamine, did not notice any influence on the dough, the volume of the loaf, or the crumb character of the bread.

Because of the importance of the patent from a social and economic point of view the author has tried to reproduce the claimed improvement. Although comparison is made in the patent to an action such as that of bromate and ascorbic acid—in other words, the influencing of the protein-proteinase system—attention was paid in the present studies to the possible activation of fermentation by the added thiamine, which could likewise cause an increase in bread volume.

Materials and Methods

A rather wide choice of flours was employed in case some types would be more susceptible than others to any effect produced by the thiamine.

a. A flour milled in a Dutch flour-mill from a grist consisting of 40% home-grown wheat, 40% foreign wheat, and 20% rye,[3] from which were separated the fractions 0–30% (patent flour A_1) and 30–67% (clear flour B_1). By mixing the fraction 0–30% with that of 30–67% in the proportion of 3:3.7, a flour (AB_1) was obtained having an extraction of 67%, which is not far short of the prewar standard in Holland.

b. A flour milled by the same mill, from a mixture consisting of 50% foreign wheat, 30% home-grown wheat, and 20% rye. The same fractions 0–30% (A_2) and 30–67% (B_2) were separated, and by mixing these fractions in the proportion 3:3.7, a flour (AB_2) of 67% extraction was compounded.

c. A flour milled exclusively from soft home-grown wheat to an extraction of about 75%.

[3] In the present situation of world wheat-shortage the millers are bound by the government to admix a certain amount of rye, mostly home-grown, to the grist.

d. A national bread-flour: "Type 10 November 1946." Composition: 50% foreign wheat, 30% home-grown wheat, and 20% rye, and extraction of 85%.

The yeast used was furnished by the Ned. Gist en Spiritusfabriek (Koningsgist).

Sodium chloride (a highly purified product) was supplied by Brocades Stheemann and Pharmacia, Meppel, and thiamine (synthetic) by the "Chemo Puro Manufacturing Company," New York.

The effect of thiamine on the protein- (gluten-) system was studied with the aid of the Brabender Extensograph, since the nature of the extensogram is known to be markedly influenced by small concentrations of such improving agents as potassium bromate and ascorbic acid. The procedure was as follows: 300 g. flour plus 6 g. sodium chloride and the thiamine, when used, were kneaded with water in the Brabender Farinograph to the normal consistency of dough (500 Brabender units). Each piece of dough was divided into two parts of 150 g. These were worked and rolled into the desired shape and set aside at a temperature of 27°C. At intervals of 45 minutes four extensograph curves were made for each piece of dough.

The influence of fermentation activity was examined with the aid of the Brabender Fermentograph as follows: The dough, prepared from flour, 2% yeast, 2% common salt, and the thiamine when used, was kneaded in the farinograph to a consistency of 450 to 500 Brabender units. Immediately after preparation an accurately weighed quantity (400 g.) was placed in the fermentograph at a temperature of 27°C. At intervals of one hour the piece of dough was well punched in accordance with the process in the bakery. The total duration of the experiment was from 4 to 5 hours; that is, about the time when the fermentation begins to weaken. With only a few exceptions, each experiment was carried out in duplicate.

The influence of thiamine on the properties of the bread was investigated by several series of baking tests. Six tinned loaves of 400 g. (each containing about 240 g. of dry substance) were baked from each dough. A dough temperature of 26° to 27°C., a proofing temperature of about 30°C., and an oven temperature of 260° to 270°C. were always maintained. Ingredients to promote diastatic power were not added, since the composition of the grain mixtures insured sufficient enzyme activity. Thiamine was added to the dough-liquor in the form of a concentrated solution by means of a pipette. Variations were introduced in the percentage of yeast, consistency of the dough, and the length of fermentation to discover the conditions most favorable to the action of thiamine on the baking behavior. The volumes of the loaves

were determined immediately after baking, and the remaining properties were estimated after about 18 hours.

Results and Discussion

The extensograph data for the 45- and 180-minute curves are summarized in Table I. Besides the highest concentration of thiamine mentioned in the patent, i.e., 6 mg. per kg. flour, an addition of 12 mg. was also examined, the greater amount thus giving a possibly vague influence of 6 mg. a chance to become evident.

For the same reason, concentrations of 10 mg. thiamine per kg. flour, as well as those of 6 mg. per kg. were examined in the fermentation experiments. Variations were also made in dough consistency and percentage of yeast. The results are summarized in Table II.

Finally the results of the different series of baking tests are collected in Table III.

The extensograms (Table I) show in a striking manner that thiamine has no effect upon the stretching properties of the dough. The curves with and without the addition of thiamine are the same at each of the

TABLE I

EFFECT OF THIAMINE ON EXTENSOGRAM MEASUREMENTS

Property measured	After 45 minutes			After 180 minutes		
	Thiamine, mg./kg. flour			Thiamine mg./kg. flour		
	0	6	12	0	6	12
FLOUR A_2 (0–30% EXTRACTION)						
Water absorption—%	52.5	52.5	52.5	52.5	52.5	52.5
Extensogram area—$cm.^2$	90.5	90.6	83.2	103.0	109.8	107.4
Max. stretching power—B. U.	370.0	365.0	340.0	540.0	545.0	540.0
Extensibility—cm.	17.5	16.6	17.0	14.4	14.7	14.5
FLOUR B_2 (30–67% EXTRACTION)						
Water absorption—%	53.5	53.5	53.5	53.5	53.5	53.5
Extensogram area—$cm.^2$	73.7	77.1	67.0	83.7	88.7	75.5
Max. stretching power—B. U.	310.0	310.0	300.0	420.0	420.0	385.0
Extensibility—cm.	16.5	16.5	16.2	14.2	14.5	14.2
FLOUR AB_2 (0–67% EXTRACTION)						
Water absorption—%	52.5	52.5	52.5	52.5	52.5	52.5
Extensogram area—$cm.^2$	82.1	84.1	83.5	100.3	103.6	99.8
Max. stretching power—B. U.	340.0	340.0	335.0	490.0	505.0	495.0
Extensibility—cm.	16.5	17.0	17.1	14.1	13.3	14.2

TABLE II

EFFECT OF THIAMINE ON THE GAS PRODUCTION OF DOUGHS OF VARYING CONSISTENCY AND YEAST CONTENT, MADE WITH FLOURS OF DIFFERENT COMPOSITION AND EXTRACTION [1]

Flour	Extractions	Yeast	Thiamine	Absorption	Farinograph consistency	Total gas production [2]	
						4 hours	5 hours
	%	%	*mg./kg.*	%	*B. U.*	*ml.*	*ml.*
A_1	0–30	2	Control	50	500	1400	—
		2	10	50	500	1440	—
AB_1	0–67	2	Control	52	450	1480	—
		2	6	52	450	1540	—
AB_1	0–67	2	Control	51	500	1475	—
		2	10	51	500	1515	—
AB_1	0–67	1	Control	53	450	765	1075
		1	6	53	450	900	1180
AB_2	0–67	2	Control	53	450	1505	1955
		2	6	53	450	1525	1980
AB_2	0–67	1	Control	53	450	790	1110
		1	6	53	450	835	1165

[1] All dough contained 2.0% of salt (flour basis). Mean values of duplicate determinations.
[2] The mean error of the mean values is about 80 ml.

testing times so that an action similar to that of bromate, ascorbic acid, or similar improvers cannot be ascribed to thiamine.

The figures derived from the fermentographic investigation (Table II) are inconclusive. The indicated influence of the thiamine on the fermentation is slight and of the same magnitude as the mean error of the mean of the duplicate determinations, but the fact that in all six series of experiments the addition of thiamine gave an increased gas-production suggests that thiamine has a positive influence on the fermentation. However, statistical calculations indicate that this influence is so slight that no definite conclusions can be drawn from it.

The question arises whether the bakers' yeast used in Switzerland, with which the experiments described in the patent in question were probably carried out, contained less thiamine than the Dutch yeast and in consequence was more sensitive to additions of this substance. However, the manufacture of bakers' yeast in West European countries is essentially the same, thus making important differences in composition very unlikely.

The baking experiments confirm the results of the dough investigation. The differences in volume of the loaves made with and without thiamine were at the most 1.5%, thus falling within the limits of experimental error. An apparent decrease in volume due to the addition of thiamine appeared in the series with the flour milled from Dutch soft wheat.

To conclude the investigation, a commercial baking test was carried out, to determine whether any differences could be obtained in the commercial bakery which uses sacks of 50 kg. of flour. Bread baked from a sack of government flour (50% foreign wheat, 30% home-grown wheat, 20% rye: 85% extraction) with thiamine added at the rate of 6 mg. per kg. flour gave similar loaves to that baked without thiamine.

TABLE III

EFFECT OF THIAMINE ON THE BAKING BEHAVIOR OF DIFFERENT FLOURS WITH VARIATIONS IN PERCENTAGE OF YEAST AND FERMENTATION TIME [1]

Procedure					Baking results				
Thiamine added per kg. flour	Yeast	Absorption	Total fermentation time	Total proof time	Shape	Texture	Loaf volume (mean)	Volume per kg. flour	Volume increase
mg.	%	%	*min.*	*min.*			*cc.*	*cc.*	%
FLOUR A_2 (0–30% EXTRACTION)									
Control	2	55	43	83	Good	Good	1720	6005	—
3	2	55	43	83	Good	Fair	1740	6070	+1.1
6	2	55	43	83	Good	Satisfactory	1740	6070	+1.1
Control	1	55	53	98	Satisfactory	Satisfactory	1530	5330	—
3	1	55	53	98	Satisfactory	Satisfactory	1530	5330	0
6	1	55	53	98	Satisfactory	Satisfactory	1530	5330	0
12	1	55	53	98	Satisfactory	Satisfactory	1550	5370	+0.8
FLOUR AB_2 (0–67% EXTRACTION)									
Control	2	55	40	93	Good	Fair	1590	5540	—
3	2	56	40	93	Good	Fair	1590	5540	0
6	2	56	40	93	Good	Fair	1590	5540	0
Control	1	56	50	103	Satisfactory	Satisfactory	1390	4810	—
3	1	56	50	103	Satisfactory	Satisfactory	1390	4810	0
6	1	56	50	103	Satisfactory	Satisfactory	1410	4880	+1.4
SOFT DUTCH FLOUR (75% EXTRACTION)									
Control	2	57.5	40	82	Moderate	Fair	1100	3760	—
3	2	57.5	40	82	Moderate	Fair	1080	3690	−2
6	2	57.5	40	82	Moderate	Fair	1095	3745	−0.5
12	2	57.5	40	82	Moderate	Fair	1085	3710	−1.5

[1] Added sodium chloride 2% (flour basis); baking time 30 minutes.

The experimental results given here show that it has not been possible to reproduce the improvement in volume of 6.8 and 15.7% respectively, on addition of 3 and 6 mg. thiamine per kg. flour, claimed in the patent in question. This applies to flour made from different grists, milled to different extractions, and baked by different formulas.

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BOOK REVIEWS

Techniques of Histo- and Cytochemistry. By David Glick. 555 pp. Interscience Publishers, New York, N. Y. 1949. Price $8.00.

As stated in the flyleaf, this book is "A Manual of Morphological and Quantitative Methods for Inorganic, Organic, and Enzyme Constituents in Biological Materials." The material presented is grouped in four parts, Microscopic Techniques, Chemical Techniques, Microbiological Techniques, and the Mechanical Separation of Tissue Components. The first two parts comprise over 90% of the text. The author has been commendably successful in bringing together in one volume the diverse methods that will serve as a very valuable aid to the research worker whose problems make it necessary to evaluate small samples of biological materials, in particular, to localize or estimate substances in the components of cells.

Appraisals and limitations of many of the procedures are given, but most of the methods are presented without evaluation. It is the author's apparent intention to describe the methods in detail and allow the investigator to use his own judgment in their application. Brief surveys of the literature relevant to the various techniques are included, together with an ample and useful bibliography. For most of the procedures the user will want to consult the original publications on the method and related subjects, and this distracts somewhat from the usefulness of the presentation of isolated, detailed instructions.

The difficult task of organizing and presenting the material is satisfactorily handled, although room for improvement is evident. For example, a historical discussion on diffusion and titrimetric methods for nitrogen and ammonia falls in the section on cuvette colorimetry. In the section on the isolation of cell nuclei, in variance with the bulk of the book and somewhat confusing to the reader, the introductory discussions are given in small print at the end of the previously presented method.

The microscopic techniques described are "those designed to establish the distribution of elements, groups, substances, or activities in microtome sections of tissues by means of examination under some form of a microscope." The section on chemical methods is prefaced by a useful discussion of the requirements and limitations of the methods. Techniques applicable to the localization of some 20 inorganic elements or radicals, 28 organic substances and groups, and 11 enzymes are presented. The section on physical methods includes techniques of fluorescence microscopy, emission and absorption histospectroscopy, microincineration, electron microscopy, and radioautography.

The material presented under the general heading of chemical techniques could be profitably consulted by anyone interested in microchemical methods. General apparatus and microchemical manipulations are described. Colorimetric methods which make use of the capillary tube technique and the microcuvette technique are

presented, followed by a good section on titrimetric techniques. The gasometric techniques described include those for micro gas analysis and for microrespirometry. Sections on dilatometric techniques and the determination of the amount of a biological sample comprise the other important portions.

The part devoted to microbiological techniques is limited to a presentation of an adaptation of the procedure for riboflavin to small volumes. The possibilities for further development in this field are deservedly mentioned.

The part on mechanical separation of cellular components is devoted to centrifugal and associated techniques for the isolation of cell nuclei, chromatin threads, cytoplasmic particles, and chloroplasts. It is these techniques together with the chemical and physical methods of analysis which R. R. Bensley in a foreword to the book regards as the "new microchemistry," which "promises to become the most important tool we possess for elucidation of the fundamental chemical pattern of protoplasm."

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Carl Alsberg—Scientist at Large. Edited by Joseph S. Davis. 182 pp. Stanford University Press, Stanford, California. 1948. Price $2.00.

Many cereal chemists will remember Carl Alsberg as one-time associate editor of this journal, as one who shared in the work of deciding the conditions of the Osborne Medal Award, and as an active worker in the field of cereal chemistry. However, Dr. Alsberg's interests extended far beyond cereal chemistry, ranging from the natural to the social sciences and the study of international relations. He was successively head of the Bureau of Chemistry, Department of Agriculture, a director of the Food Research Institute at Stanford University, and director of the Giannini Foundation for Research in Agricultural Economics at the University of California.

This book is edited by J. S. Davis who collaborated with Dr. Alsberg at the Food Research Institute, Stanford University. Five of Dr. Alsberg's former colleagues, A. L. Kroeber, D. D. Van Slyke, F. B. Linton, R. D. Calkins, and J. B. Condliffe, have each written a biographical chapter covering the various aspects of his career.

These chapter titles are, respectively, The Making of the Man, Work in the Natural Sciences, Chief of the Bureau of Chemistry, University Professor and Administrator, and Social Scientist Beyond the University. Besides these fine tributes to an outstanding man, the book contains three of Dr. Alsberg's own stimulating papers, namely, "Progress in Chemistry and the Theory of Population," "What the Social Scientist Can Learn from the Natural Scientist," and "Commencement Address at Reed College."

The book also includes a classified bibliography of Alsberg's publications.

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Biochemical Preparations. Volume I. Edited by Herbert E. Carter. XI + 76 pp. John Wiley and Sons, Inc., New York, N. Y. 1949. Price $2.50.

This is the first of a projected series of volumes describing the preparation of compounds of biochemical origin or interest. It is the aim of the editorial and the advisory boards to cover those compounds which are not offered commercially but which can be prepared from available starting materials. In general, isolation methods will be emphasized, but if certain useful compounds can be obtained by synthesis, the latter will be used. It is also planned to include techniques of general application.

Like the practice followed by *Organic Syntheses* and *Inorganic Syntheses*, the contributors have made their directions for the preparations very specific and the procedures have been checked in an independent laboratory. The 16 procedures describe the preparation of 19 compounds.

Of interest to the enzyme chemist, isolation methods are given for adenosinetriphosphate (ATP), adenosinediphosphate (ADP), and diphosphopyridine nucleatide (DPN), as well as for α-glucose-l phosphate and for D,L-glyceraldehyde-3

phosphoric acid. In the field of the amino acids, L-serine and L-alanine are prepared from silk, with tyrosine and glycine as by-products. For their separations, three more strictly organic compounds are needed for which methods of preparation are given. A preparation of L-lysine hydrochloride from a protein hydrolyzate is given as well as the isolation of dopa from the Georgia velvet bean. Glutamine is prepared from beets and the biological aspect is emphasized when the method points out how the content of glutamine may be increased by the application of ammonium sulfate to the growing beets. In the field of protein isolations, a method for obtaining casein from milk is outlined together with two methods for isolating lysozyme from egg white.

The racemization of an amino acid through the azlactone is described for tyrosine, the resolution of the mixture is achieved through the fractionation of the brucine salts of acetyl D,L-tyrosine. The lipide field is represented by an isolation of lycopene; this involves the technique of chromatography.

A worth-while feature incorporated into this initial issue is a section under each preparation on the properties and the purity of the product. Included is a list of 58 preparations in *Organic Syntheses* which are of biochemical interest. The manual conforms to the high standards set by the sister series for organic and inorganic syntheses and the editorial board is to be commended for its efforts. Readers of this journal will no doubt find the preparative methods in this and succeeding issues of great help when they wish to study isolated biochemical systems.

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Aquametry. Application of the Karl Fischer Reagent to Quantitative Analysis Involving Water. Volume V. Chemical Analysis. J. Mitchell, Jr., and D. M. Smith. 51 illus. 143 tables. 456 pp. Interscience Publishers, Inc., New York. 1948. Price $8.00.

This fifth volume of a series of monographs on analytical chemistry is a comprehensive treatment of the application of the Karl Fischer reagent to quantitative analyses involving water. The authors, who are both from the laboratories of E. I. duPont de Nemours Co., Inc., have coined the term "aquametry" to denote the analytical process of measurement of water; the content of this book is, however, more accurately described by the subtitle.

A short introductory chapter of 15 pages is devoted to a brief review of the various methods of determining water. The remaining 245 pages of Part I discuss fully the application of the Karl Fischer reagent to the determination of water. This discussion comprises the history, chemical background, and the various analytical techniques which include macro, micro, visual, electrometric, and other procedures. This is followed by a discussion of the application of the Karl Fischer method to the determination of water in organic and inorganic chemicals and industrial products. Of interest to cereal chemists will be the sections on moisture determination in foodstuffs, carbohydrates, and edible oils. Data are given on wheat, barley, wheat flour, oat flour, corn flakes, cottonseed, and similar products. Finally Part II, comprising 134 pages, deals with organic reactions in which water is liberated or consumed and with the determination of organic functional groups with the Karl Fischer reagent.

This book is essentially a laboratory text in which the various procedures are described in sufficient detail so that only occasional reference to original literature would be necessary. Its chief merit lies in the service which the authors have done by bringing together the voluminous literature which has appeared since the original discovery of the Karl Fischer reagent in 1935. In the opinion of the reviewer, the value of this monograph would have been enhanced had the authors allotted more space to the discussion of the basic methods of water determination, and perhaps less to the development of the less well-established applications of the Karl Fischer method.

The paper, typography, and binding are good. The book is written in a clear and logical manner, and is almost free from errors.

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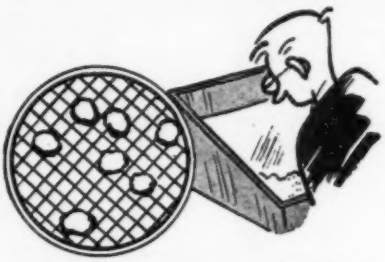

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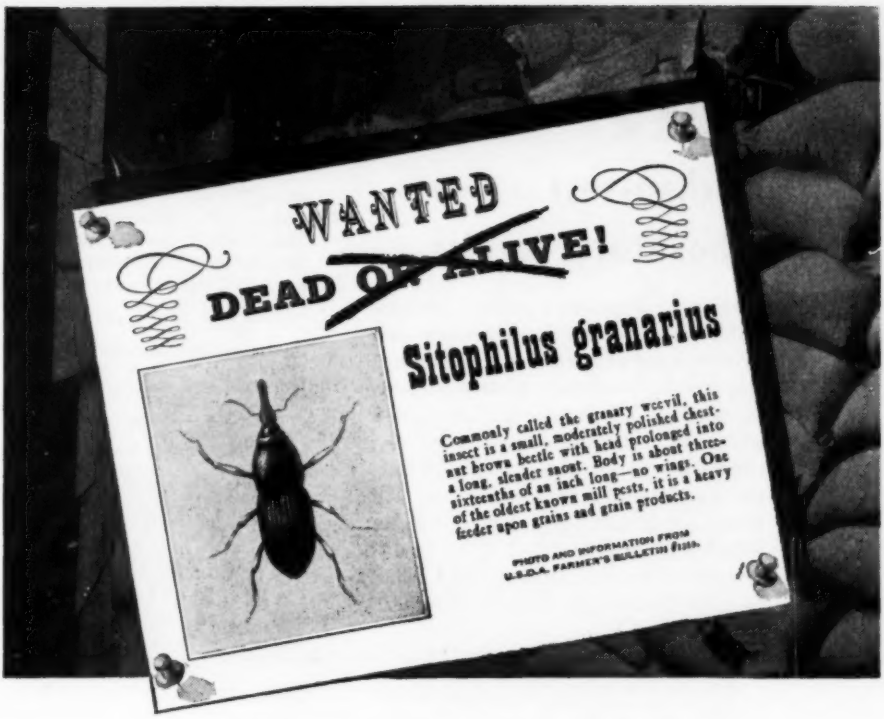
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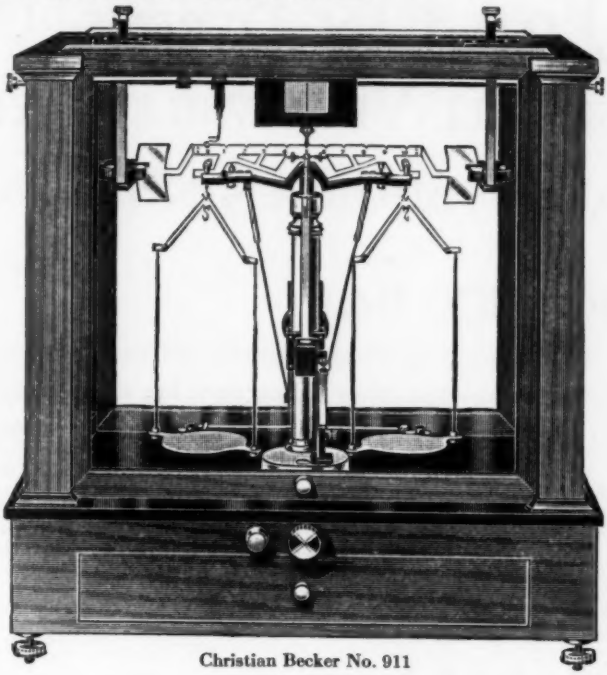

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